

Dharam P. Abrol

Asiatic Honeybee *Apis cerana*

Biodiversity Conservation and
Agricultural Production



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Biodiversity Conservation and Agricultural
Production

 Springer

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Dedicated to, almighty God for granting me life, strength and holding my hand to reach where I stand now, to my parents for their eternal blessing from heaven. To my Guru Late Professor R. P. Kapil—an excellent scientist and splendid person who introduced me to the fascinating world of honeybees. To my wife Professor Asha—for her enduring love, encouragement, support and gifted sense of humor. To my daughter Vitasta and son Rajat for their revolutionary and innovative ideas.

Foreword

The world is facing food deficit that is exacerbated by escalating prices, coupled with instability of climatic cycles. The growing population pressure has hastened the environmental degradation, ultimately posing a threat to natural resources and fast approaching famine. In the next 50 years, the global population is expected to reach 9 billion; thus doubling the food, feed and crop demand. Concomitantly, this situation has further aggravated because of pollinator decline worldwide resulting in pollination crisis. These problems have been further aided and abetted by a lack of prophylactic progress in the conservation of biodiversity and increased agricultural production.

Amongst pollinators, honeybees are declining at alarming rate. The European honeybee *Apis mellifera* is a well-studied insect. On the other hand the Asiatic honeybee *Apis cerana* which has provided mankind with invaluable products, conservation of natural resources and pollination of agricultural crops is relatively little understood. It is the most valuable natural resource of beekeeping and has been considered a vital component of the natural ecosystem, well adapted to the local climatic conditions and floral resources through centuries of natural selection. Despite its economic usefulness, biodiversity of Asian hive bee *A. cerana* is suffering precipitous decline and is threatened with extinction in its entire native habitat.

To promote beekeeping as a sustainable option for rural development, crop production and biodiversity conservation, there is an urgent need to generate information on this important species. Although a number of publications have appeared on honeybees in the market, no attempt has been made to approach the subject in a systematical and a comprehensive manner in case of *A. cerana*. Although a brief perusal of the studies on *A. cerana* at the website Google scholar shows that during the last century the publication of research papers in this area has grown exponentially, and in just the last decade, some 5,000 items have appeared. In the event, it is both fair and pertinent to ask: "Is there actually a need for yet another work on *The Asiatic Honeybee Apis cerana: The Conservation of Biodiversity, and Agricultural Production*"? The answer is yes. As void exists on different facets of biological conservation, agricultural production and role of *A. cerana* in improving food security and livelihoods. Professor Dharam P. Abrol attempts to fill this gap by providing detailed information on

different aspects of *A. cerana* leading to biodiversity conservation and sustainability of food production.

The history of beekeeping with *A. cerana* in Asia is at least as ancient as that of beekeeping with *A. mellifera* in southern Europe and the Middle East. *A. cerana* and *A. mellifera* are two related species of cavity-nesting honeybees that are thought to have diverged 2–3 million years ago. Each species can decode the information contained in the waggle dances of the other. *A. cerana* as compared with the European honeybee *A. mellifera* forages on a wide variety of resources. Even the resources that provide small quantities of rewards are worked upon resulting in outcrossing of a large spectrum of crops and wild flowers.

The population of *A. cerana* and other pollinators are declining at an alarming rate that has threatened the existence of plant life and this downward trend could damage dozens of commercially important crops. A decline in pollinator populations is one form of global change that actually has credible potential to alter the shape and structure of terrestrial ecosystems. The decline in pollinator population and diversity presents a serious threat to agricultural production, conservation and maintenance of biodiversity in many parts of the world.

A. cerana is a species that has shown great adaptive potential, as it is found almost everywhere in highly diverse climates. There is enough evidence to suggest that environmental changes have a direct influence on honeybee development. Asian honeybee *A. cerana* can defend attack of enemies with the use of various weapons including stings, mandibles, legs and wings—variously used to grasp, pull, bite and sting. The outstanding effectiveness of nest defense in honeybees is not so much based on the faculties of individual bees, but on the social coordination of thousands of individuals.

Interspecific competition for a limited resource can result in the reduction of survival, growth and/or reproduction in one of the species involved. Likewise, introduction of *A. mellifera* eliminated *A. cerana japonica* in China and Japan and *A. cerana indica* in Indian subcontinent including India, Pakistan, Nepal, Bangladesh and other neighbouring countries. Negative impacts of alien bees need to be carefully assessed before further introductions are carried out. The main objective of this book is to encourage beekeeping interventions helping people to strengthen livelihood and ensure maintenance of habitat and biodiversity. This is, indeed, an awesome task and we must congratulate Professor Dharam P. Abrol for bringing out this book for the benefit of the global community.



Dated 21, March 2013

Prof. Dr. Michal Woyciechowski

Preface

Asiatic honeybee *Apis cerana*, are small honeybees of southern and southeastern Asia such as China, India, Japan, Malaysia, Nepal, Bangladesh and Papua New Guinea. This species is the sister species of *Apis koschevnikovi*, and both are in the same subgenus as the European honeybee, *Apis mellifera*. For ages, colonies of the oriental honeybee *A. cerana* have provided mankind with honey and beeswax, as well as furnishing invaluable service in the pollination of agricultural crops. This bee's range of distribution is far greater than those of *A. florea* and *A. dorsata*: it is found throughout the tropical, sub-tropical and temperate zones of Asia, occurring in the Indian sub-continent and Sri Lanka in the west, through Southeast Asia, to Indonesia and the Philippines in the east. Further north, it is found in the southern USSR and China, through the Korean peninsula, to Japan. This wide range has led to important variations amongst the bee's geographical races: particularly between the tropical and temperate races, there are wide differences in workers' body size, nest size, colony population, swarming and absconding behaviour. The temperate and sub-tropical races appear to store greater quantities of food than the tropical races, which in turn are more mobile than the former, tending to swarm, abscond and migrate quite frequently. Furthermore, *A. cerana* is adept in collecting sporadic nectar flowers in disturbed or extensively modified habitats in the mountain and forest region and can thermoregulate between 33 and 35.5 °C in a temperature range of 12–36 °C.

A. cerana, the indigenous hive bee of Asia, is the most valuable natural resource of beekeeping and has been considered a vital component of the natural ecosystem. It is well adapted to the local climatic conditions and floral resources through centuries of natural selection. It has been reported to be an excellent pollinator of crops that bloom in early spring such as almonds, apples, pears, plums and different vegetable seed crops. Beekeepers and pollination scientists have been experiencing rapid decline in *A. cerana* populations, which may result in the loss of plant biodiversity in an area and create socio-economic problems. Because of the decline of *A. cerana* colonies in many regions of Asia, *A. cerana* is an endangered species. The smaller the native population of *A. cerana* in any area, the higher the danger for this bee because of its mating behaviour. When the *A. cerana* population is destroyed, a native and well-adapted pollinator for both native and agricultural plants will be lost. The results for

loss of native plant biodiversity and the pollination of agricultural crops cannot be estimated because *A. mellifera* is not able to pollinate as effectively as *A. cerana*?

A. cerana offers several comparative advantages over *A. mellifera* as pollinator. These include initiation of early foraging at lower temperatures, longer foraging hours, shorter flight range, no competition for food and nesting sites with other bee species, co-evolution with native crops, more suitable for glass house pollination, better searching ability for sparse floral resources. Moreover, this bee species is more docile and industrious in nature, less prone to attacks of wasps, and a high level of resistance to nosema disease and parasitic mites. *A. cerana* can coexist with other native bee species and require little chemical treatment of colonies to control epidemics. However, as yet, this native bee species has not become popular amongst beekeepers because of several behavioural characteristics. These include their frequent swarming and absconding, their tendency to rob, their production of a large number of laying workers, and their lower honey yields. These negative traits show eco-geographical variations depending upon the sub-species/geo-ecotypes and management efficiency of the beekeepers and are amenable through basic and action research.

Despite its economic usefulness, biodiversity of Asian hive bee *A. cerana* is suffering precipitous decline and is threatened with extinction in its entire native habitat. For example, in Japan, beekeeping with this native bee species has been completely replaced by European honeybee, *A. mellifera* and only a few beekeepers and research institutes are raising *A. cerana* colonies. In China, out of more than 8.5 million colonies of bees kept in modern hive, 70 % are exotic *A. mellifera*. Similarly, in South Korea, only 16 % beekeeping is with native *A. cerana* and remaining has been replaced by exotic *A. mellifera*. Similarly in India, only 10 % of beekeeping is done with *A. cerana*.

During the past four decades, human population has increased more than twofolds exerting a tremendous pressure on the natural resources and the land especially for food, fuel and timber. As a consequence, vast forests have been converted into agricultural land and mountains have become barren due to ruthless cuttings and grazing, thus extensively destroying the food and habitat of several pollinators species. Along with these, use of chemicals, too, have greatly wiped out the population of natural pollinators, thus resulting in failure of reproduction in several cross-pollinated plant species including the agricultural crops. This book on *A. cerana*, addresses two basic questions. How the *A. cerana*, can be utilized in the conservation of plant species? How the Asiatic honeybee *A. cerana*, can be utilized for sustainable agriculture and securing livelihood without disturbing the environment and the natural balance?

This book emphasizes conserving and culturing Asiatic honeybee *A. cerana*. It provides complete information on all aspects of *A. cerana* beekeeping. It is first of its kind which deals in details on biology, biogeography, reproduction, genetics, molecular phylogeny, interaction with other species, floral resources, dance language, safety from pesticides, management problems, loss of genetic diversity, behavioural defence, role in food production, livelihood security and conservation strategies for

protecting biodiversity and enhancing crop productivity. Despite its economic usefulness, biodiversity of Asian hive bee *A. cerana* is suffering precipitous decline and is threatened with extinction in its entire native habitat.

To promote beekeeping as a sustainable option for rural development, crop production and biodiversity conservation, there is an urgent need to generate information on this important species. Although a number of publications have appeared on honeybees in the market, no attempt has been made to approach the subject in systematically and in a comprehensive manner in case of *A. cerana*. The aim of this book is to fill the gap by providing detailed information on different aspects of *A. cerana* leading to sustainability and environmental protection. The compilation of this book is unique in the sense that in the context of pollinator decline over the world, conservation of this species will be a step for sustaining food security.

A vast spectrum of people has helped in one way or the other in the writing of this book, which would have remained a distant dream without their active help and support. This book is the outcome of my personal experiences and the contributions of several workers which have been incorporated. I express my humble and profound thank to all of them whose hard work has enabled me to compile the suitable information in a such a manner that it would be useful to those interested in basic and applied pollination. The illustrations and figures are either original or redrawn from other sources which have been cited individually in the figure legends. All the authors whose work has been used/refereed deserve special appreciation and heartiest acknowledgments and if, any omission has crept anywhere I shall be responsible and apologize in advance.

I am particularly thankful to Professor Dr. Raghavendra Gadagkar, Centre for Ecological Sciences, Indian Institute of Sciences Bangalore who has always been a source of inspiration, needed help, guidance and encouragement. I thank my university authorities for the excellent working atmosphere and needed encouragement for compiling such a voluminous book. Dr. Uma Shankar deserves special appreciation for redrawing many of the line drawings. Dr. Debjyoti Chatterjee needs special mention for painstaking job of preparing index. I am also extremely thankful to Zuzana Bernhart Senior Publishing Editor and Elisabete Machado Editorial Assistant of Springer who took great pains and keen interest in publication of this book in a very impressive way. Words are insufficient to express my gratitudes to Dr. D. K. Arora Hon'ble Vice-chancellor for his encouragement and inspiration.

Last but not the least, my sincere thanks are due to my wife Professor Dr. Asha Abrol, daughter Er. Vitasta and son Er. Rajat for their endurance and help while writing this book.

Jammu, March 31, 2013

Dharam P. Abrol

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About the Author



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1999–2000—a prestigious National Award from Indian Council of Agricultural Research New Delhi for his Hindi book on beekeeping entitled *Madhmakhi Palan: Sidhant Evam Vidhian* and 11th Asian Apicultural Association Award—2010 for outstanding contributions in Apiculture. He is presently working in Division of Entomology Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu as Professor and Head and engaged in teaching and research in Entomology, which he has been doing more than last 27 years.

Chapter 1

Introduction

1.1 Species Diversity of *Apis* in Southeast Asia

There are about 20,000 species of bees belonging to the superfamily Apoidea. *Apis* honeybees belong to a small subgroup of this superfamily comprising nine species and *A. cerana* is one of five cavity nesting species (Koeniger et al. 2010). Of critical importance is the recognition of the genetic diversity present within *A. cerana*. Initial studies on the species refer to races, strains, and subspecies (Ruttner 1988). More recent studies note that this species may be subject to cryptic speciation and that its taxonomy is by no means resolved (Oldroyd and Wongsiri 2006). Three variants, once thought to be members of *A. cerana*, are now recognized as distinct species (e.g., *A. nigrocincta*, *A. koschevnikovi*, and *A. nuluensis*) and other species may yet be recognized (Gloag et al. 2010). Therefore, to avoid confusion between current recognized variants of *A. cerana* (e.g., *indica*, *japonica*, *javana*), they will be referred to simply as “genotypes” from particular locations.

Before continuing the description and analysis of *A. cerana* from existing studies, a caveat should be introduced at this point. Care should be taken when interpreting aspects of the biology and ecology of *A. cerana* reported in the literature. This is simply because what is known about the bee comes from particular places and times. That information clearly shows that *A. cerana*, like *Apis mellifera*, exhibits a great deal of plasticity in its biology and ecology across its geographical range. Thus, the biology and ecology attributed to *A. cerana* at one location in Asia does not mean that those attributes will apply to *A. cerana* at other locations.

Honeybees have settled almost all over the planet. They live both in regions with cold climates and long severe winters and in the tropics where winters never occur and the summer temperatures are usually higher. Bees’ adaptability to different climates and environments has proved to be genuinely amazing. As a result of specific climatic conditions and peculiarities of nectariferous flora, there developed various breeds of honeybees during the course of their evolutionary history. Based on essentially morphological and behavioral analyses and the aid of different genetic techniques, the classification systematic of the true honeybees has obtained great achievements in the last two decades of the twentieth century. The number of recognized honeybee species has been reduced since the descriptions of Maa (Maa 1953), because many types are now seen as subspecies (Otis 1997).

The tribe, Apini, consists of only one small monophyletic genus, *Apis* that comprises nine honeybee species: *A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nigrocincta*, *A. nuluensis*, *A. dorsata*, *Apis laboriosa*, *A. florea* and *Apis andreniformis* (Otis 1997; Tingek et al. 1996). Out of these nine species, the five initial species nest in cavities that have a number of combs. The last four nest in the open and have a single comb. *Apis* species are divided into three lineages: the cavity nesting bees, *A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nigrocincta*, and *A. nuluensis*; and open nesting the dwarf bees, *A. florea* and *A. andreniformis*; the giant bees, *A. dorsata* and *A. laboriosa*. Of the nine species, only *A. mellifera* and *A. cerana* have been “domesticated” for a long time (Koeniger 1976a). *A. mellifera* is the most studied and economically exploited species. All *Apis* species, except for *A. mellifera*, are native to southeast Asia. This region is a center of *Apis* diversity and makes scientists pay great attention to the recently recognized species such as *A. nigrocincta* and *A. nuluensis*.

1.2 Origin and Distribution of the Genus *Apis*

Honeybees as a group appear to have their center of origin in southeast Asia (including the Philippines), as all but one of the extant species are native to that region, including the most primitive living species (*A. florea* and *A. andreniformis*). The first *Apis* bees appear in the fossil record in deposits dating about 40 million years ago during the Eocene period; that these fossils are from Europe does not necessarily indicate that Europe is where the genus originated, as the likelihood of fossils being found in southeast Asia is very small, even if that is the true origin. At about 30 million years before present, they appear to have developed social behavior and structurally are virtually identical with modern honeybees. Among the extant members of the genus, the more ancient species make single, exposed combs, while the more recently evolved species nest in cavities and have multiple combs, which greatly facilitated their domestication.

The earliest definitive members of the tribe Apini are known from the Oligocene of France and Germany. These comprise *Apis henshawi* Cockerell, and under some classificatory schemes *Apis vetusta* Engel, from Rott and Enspel, Germany (e.g., Cockerell 1907; Statz 1931, 1934; Engel 1998, 1999; Wedmann 2000), and *Apis cuenoti* Théobald from Céreste, France (Théobald 1937; Nel et al. 1999), the latter of which is sometimes considered a synonym of *Apis henshawi* (Engel 1999). Generally, the forewing venation of these Oligocene honeybee populations resembles that of Recent *A. dorsata* Fabricius, but the species are distinctly smaller, more typical of the average-sized *A. mellifera* and *A. cerana* Fabricius (Nel et al. 1999; Wedmann 2000). Apini from the Oligocene and Miocene are known from Spain and France (e.g., Arillo et al. 1996; Nel et al. 1999), Italy (Handlirsch 1907), Germany (Zeuner 1931; Pongrácz 1931; Armbruster 1938a; Prokop and Fikáček 2007), Austria (Nel et al. 1999), the Czech Republic (Říha 1973; Prokop and Nel 2003), China (Hong 1983; Zhang 1989, 1990), Japan (Engel 2005, 2006), and most surprisingly the

western USA (Engel et al. 2009). For most of the Late Oligocene and Miocene forms, the specific status remains questionable (Nel et al. 1999; Engel 2006). There are no unquestionable fossils of *Apis* from the Pliocene, and only records of modern *A. mellifera* in east African copal of Late Pleistocene or younger age (e.g., Foord 1890; Cockerell 1909; Zeuner and Manning 1976) as well as petrified combs of *A. cerana* from the Malay Peninsula (Stauffer 1979). Among all these records, the honeybees from Rott and the Randeck Maar in Germany are the most abundant, particularly from the latter deposit.

William Scheuthle was the first to discover honeybees at the Randeck Maar (Early Miocene, southwest Germany) in 1926. In 1928, William Scheuthle and Ludwig Armbruster, a prominent apiculturist of the day, excavated more material. Finally, in 1938, the accumulated material was first formally described based on an examination of 72 specimens (Armbruster 1938a, b, c). Armbruster (1938a) classified the material into three species of a then new genus, dubbed *Hauffapis*, although he himself pointed to the obvious similarities of *Hauffapis* to *Apis* and especially to recent *A. dorsata* and the contemporaneous fossil species *Apis armbrusteri* Zeuner from the nearby Böttingen Marmor (Zeuner 1931). The generic name *Hauffapis*, unfortunately, was not validly proposed and so is not nomenclaturally available (Michener 1990, 1997; Engel 1999). Armbruster (1938a) also noted that some specimens resembled *A. mellifera* in terms of forewing venation (vide infra), which further convinced him that he was dealing with multiple species and which he named *Hauffapis scheuthlei*, *Hauffapis scheeri* and *Hauffapis scharmanni* (naming them for his collecting partners, along with various infraspecific forms). Subsequently, Zeuner and Manning (1976) united all these taxa, including that from Böttingen Marmor, into a single species and under the name *A. armbrusteri*, considering Armbruster's three forms to be separate subspecies. The fossil bees from the Böttingen Marmor are preserved only as hollow imprints and, while they can be attributed to *Apis*, many features remain unknown from the type series (Zeuner 1931; Zeuner and Manning 1976).

The abundance of material from Randeck Maar represents a wonderful opportunity to evaluate more critically these fossil honeybees, since from most localities only one or a very few specimens are typically available. Unfortunately, several of the diagnostics used for the determination of extant *Apis* species or subspecies cannot be used for the differentiation of fossil Apini, even when excluding the obvious biochemical attributes. For example, *A. cerana*, *A. mellifera*, and their subspecies, along with *A. koschevnikovi* Enderlein and *A. nigrocincta* Smith, are generally recognized from differences in size, coloration of setae and integument, distribution and proportions of setal bands on the metasoma, length of the proboscis, sternal and leg podite proportions, the presence or absence of a distal abscissa to M in the hind wing (absent in *A. mellifera*), structure of the drone legs and endophallus, and behavioral aspects such as the time of drone mating flights, structure of brood cell caps, or the position of a worker while wing flapping in front of the hive (e.g., Ruttner 1988; Verma et al. 1994a; Hadisoesilo and Otis 1996, 1998; Damus and Otis 1997; Sheppard et al. 1997; Sheppard and Meixner 2003; Radloff et al. 2010, 2011). Several of these are highly variable (e.g., size, coloration, time of drone flights), while more

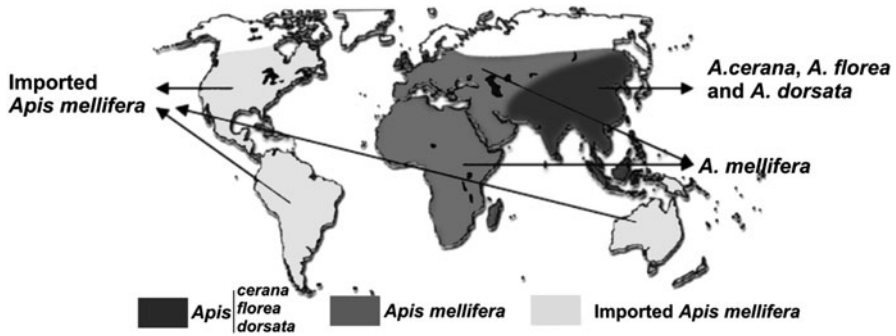


Fig. 1.1 Origin and distribution of various species of honeybees and movements of *A. mellifera*

consistent traits such as those from the hind wing are infrequently preserved in fossil *Apis*. Moreover, behavioral aspects are rarely detectable in the fossil record unless they leave a discrete trace or physical structure suitable for fossilization (e.g., traces of leaf-cutter bees (Wappler and Engel 2003; Wedmann et al. 2009), fossilized nests (Stauffer 1979). To date, no fossil of a drone honeybee has been recovered and, indeed, male bees of any tribe or family are exceptionally rare as fossils (e.g., Camargo et al. 2000; Engel 2001a; Hinojosa-Díaz and Engel 2007). Thus, using only the typical criteria for segregating species such as *A. cerana*, *A. mellifera*, or their relatives, and particularly subspecies within each of these forms, it would be nearly impossible to distinguish these taxa in the fossil record. This has greatly hampered any understanding of fossil *Apis*.

1.3 Recent Honeybee Species

The number of Recent species of *Apis* and their respective diagnoses has been a matter of debate over the last couple of decades. Interpretations vary between six or seven species on the conservative end (Alexander 1991a, b; Engel and Schultz 1997; Engel 1999; Fig. 1.1) and 10 or 11 (Arias and Sheppard 2005; Lo et al. 2010), or even as many as 24 (Maa 1953) at the higher extreme. Most of the controversy surrounds the status of some southeast Asian populations (Koeniger et al. 2010; Radloff et al. 2011). Although several analyses have examined *Apis* phylogeny, most recent investigations have relied solely on DNA sequence data and sometimes with exceptionally small samples across the diversity of honeybee populations (e.g., Willis et al. 1992; Tanaka et al. 2001; Arias and Sheppard 2005; Raffiudin and Crozier 2007; Lo et al. 2010). Only one analysis has synthesized data from multiple sources—adult morphology, larval morphology, DNA sequences, and behavior (Engel and Schultz 1997). The species recognized in the Engel and Schultz (1997) combined analysis were *A. mellifera*, *A. florea* Fabricius, *A. andreniformis* Smith, *A. koschevnikovi*, *A. cerana*, and *A. dorsata* (these authors did not consider *A. nigrocincta* specifically distinct from *A. cerana* at that time). *A. nigrocincta* was subsequently added to this list of honeybee

diversity (Hadisoesilo et al. 1995; Hadisoesilo and Otis 1996, 1998; Engel 1999; Smith et al. 2000, 2003; Fig. 1.1). Although the species recognized in the diversity of phylogenetic treatments varies under the biological, phylogenetic, or evolutionary species concepts, there remains broad congruence as to the principal clades within the genus and their interrelationships (e.g., Engel and Schultz 1997; Engel 1998, 1999, 2006; Leelamanit et al. 2004; Arias and Sheppard 2005; Raffiudin and Crozier 2007; Lo et al. 2010). These studies agree that the lineage of dwarf honeybees, *A. florea* and *A. andreniformis*, diverged early on from the remainder of recent *Apis* clades, with the giant honeybees, *A. dorsata* and its predecessors, diverging from the common ancestor of a clade comprising *A. mellifera* and the “*cerana*” group of species (i.e., *A. cerana*, *A. koschevnikovi*, *A. nigrocincta*). These three groups are sometimes accorded subgeneric status as *Micrapis* Ashmead, *Megapis* Ashmead, and *Apis* s.str. (e.g., Engel 1999, 2001b, 2002, 2006; Engel et al. 2009; Koeniger et al. 2011), although some less widely employed classifications have considered them as separate genera in their own right (e.g., Ashmead 1904; Maa 1953; Wu and Kuang 1987). *A. mellifera* is the most widespread of these species, occurring throughout Europe, Africa, Northwestern Asia (e.g., Ponto-Caspian and as far east as the Tien Shan), the Levant, Caucasia, and the Iranian Plateau (Ruttner 1988, 1992; Ruttner et al. 1985; Sheppard and Meixner 2003), as well as adventive in the Americas and Australia (e.g., Kerr 1957; Sheppard 1989; Engel 1999; Moritz et al. 2005). The remaining recent honeybees are largely restricted to Asia (Michener 2007; Radloff et al. 2011), with the exception of *A. florea*, which is known also from Jordan, the eastern Arabian Peninsula, and northeastern Africa (Lord and Nagi 1987; Mogga and Ruttner 1988; Engel 1999; Michener 2007; Dathe 2009; Haddad et al. 2009; Moritz et al. 2010). The precise distributions of the remaining Asian species and morphotypes are summarized by Otis (1996), Engel (1999), Oldroyd and Wongsiri (2006), and Hepburn and Radloff (2011).

1.4 Honeybee Species

Among domesticated honeybees, *A. mellifera* Linnaeus is widely distributed and most commonly recognized native to Africa and Europe, and subdivided into about 24 subspecies followed by the eastern Asiatic hive bee *A. cerana*, which is distributed throughout Asia serving as a great source of food security, sustainability, and livelihood security.

1.4.1 Eastern Honeybee, *A. cerana* F., 1793

Apis honeybees belong to a small subgroup of superfamily Apoidea comprising nine species and *A. cerana* is one of the five cavity nesting species (Koeniger et al. 2010). Of critical importance is the recognition of the genetic diversity present within *A. cerana*. Initial studies on the species refer to “races,” “strains,” and subspecies

(Ruttner 1988). More recent studies note that this species may be subject to cryptic speciation and that its taxonomy is by no means resolved (Oldroyd and Wongsiri 2006). Three variants once thought to be members of *A. cerana* are now recognized as distinct species (*A. nigrocincta*, *A. koschevnikovi*, and *A. nuluensis*) and other species are as yet to be recognized (Gloag et al. 2010).

A. cerana, or the Asiatic honeybee (or the Eastern honeybee), are small honeybees of southern and southeastern Asia, such as China, India, Japan, Malaysia, Nepal, Bangladesh, and Papua New Guinea. This species is the sister species of *A. koschevnikovi*, and both are in the same subgenus as the European honeybee, *A. mellifera*.

For ages, colonies of the oriental honeybee *A. cerana* have provided mankind with honey and beeswax, as well as furnishing invaluable service in the pollination of agricultural crops. This bee's range of distribution is far greater than those of *A. florea* and *A. dorsata*: it is found throughout the tropical, subtropical, and temperate zones of Asia, occurring in the Indian subcontinent and Sri Lanka in the west, through southeast Asia, to Indonesia and the Philippines in the east. Further north, it is found in the southern USSR and China, through the Korean peninsula, to Japan. This wide range has led to important variations among the bee's geographical races: particularly between the tropical and temperate races, there are wide differences in workers' body size, nest size, colony population, and swarming and absconding behavior. The temperate and subtropical races appear to store greater quantities of food than the tropical races, which in turn are more mobile than the former, tending to swarm, abscond, and migrate quite frequently.

In the wild, the oriental honeybees construct their multiple-comb nests in dark enclosures such as caves, rock cavities, and hollow tree trunks. The normal nesting site is, in general, close to the ground, not more than 4–5 m high. The bees' habit of nesting in the dark enables man to keep them in specially constructed vessels, and for thousands of years *A. cerana* has been kept in various kinds of hives, i.e., clay pots, logs, boxes, wall openings, etc. Despite the relatively recent introduction of movable-frame hives, colonies of *A. cerana* kept in traditional hives are still a common sight in the villages of most Asian countries. As a result, the feral nests of the oriental honeybee in tropical Asia sustain fewer casualties in being hunted by man than those of the dwarf and giant honeybees.

The several combs of an *A. cerana* colony are built parallel to each other, and a uniform distance known as the "bee space" is respected between them. The body size of the workers of this bee is much smaller than that of the *A. dorsata* workers, and its brood comb consists of cells of two sizes: smaller for the worker brood and larger for the drone brood. The queen cells are built on the lower edge of the comb. As in the other *Apis* species, honey is stored in the upper part of the combs, but also in the outer combs, adjacent to the hive walls.

Following the invention of the movable-frame hive for the European honeybee about a century ago, traditional beekeeping with *A. cerana* has been partially replaced by this modern method in several Asian countries, and at the same time attempts have been made with varying degrees of success to improve hiving techniques and colony management. These beekeepers are found across the breadth and range of the Asian countries. There are rural beekeepers in the high mountains of the Himalayas who



Fig. 1.2 Nesting sites of *A. cerana*

keep log hives in house walls and revetments. Each family typically owns half a dozen bee-logs and honey combs are only removed for local consumption.

In the wild, *Apis cerana* prefer to nest in small spaces, such as hollowed out tree trunks (Fig. 1.2). Like the European honeybee, they are sometimes domesticated and used in apiculture, mostly in wooden boxes with fixed frames. Their size is similar or somewhat smaller than *A. mellifera*, and they also have a more prominent abdominal stripes. Their honey yield is smaller, because they form smaller colonies. Their beeswax is used to treat and heal wounds. *A. cerana* is the natural host to the

mite *V. destructor*, a serious pest of the European honeybee. Having coevolved with this mite, *A. cerana* exhibits more careful grooming than *A. mellifera*, and thus has an effective defense mechanism against *Varroa* that keeps the mite from devastating colonies. Other than defensive behaviors such as these, much of their behavior and biology (at least in the wild) is very similar to that of *A. mellifera*. They have a strong thermal defense: When their hive is invaded by the Japanese giant hornet (*Vespa mandarinia*), about 500 Japanese honeybees (*A. cerana japonica*) surround the hornet and vibrate their flight muscles until the temperature is raised to 47 °C (117 °F), heating the hornet to death, but still under their own lethal limit (48–50 °C).

The Asiatic hive honeybee, *A. cerana*, is widespread in temperate and tropical Asia (Smith et al. 2000). *A. cerana* provides honey, beeswax, and the invaluable service of crop pollination. They tend to swarm, abscond, and migrate quite frequently (Akranakul 1976; Maa 1953; Morse and Moch 1971; Otis 1990; Richards 2001; Smith et al. 2000; Wongsiri et al. 1996; Wu and Kuang 1987). Among the native bees of Asia, *A. cerana* will likely become an increasingly important beekeeping resource. Its nest structure is similar to that of *A. mellifera* because it builds multiple combs inside a nest cavity. It also performs waggle dances inside the nest, usually in the dark, like *A. mellifera* (Lindauer 1956). As *A. cerana* has not been domesticated to the same extent as *A. mellifera*, it poses some problems for apiculture such as high swarming and absconding rates, a different set of parasites, and more limited honey storage capabilities. However, they are strongly resistant to *V. jacobsoni* and predatory wasps (Kerr et al. 1974; Ruttner 1988; Wongsiri et al. 1996a). In the wild, these bees construct their nests in dark enclosures such as caves, rock cavities, and hollow tree trunks. The normal nesting site is usually close to the ground, not more than 4–5 m high. The bees' habit of nesting in the dark enables one to keep them in specially constructed vessels. For thousands of years, *A. cerana* has been kept in various kinds of hives (clay pots, logs, boxes, wall openings, etc). Despite the relatively recent introduction of Langstroth-frame hives, colonies of *A. cerana* kept in traditional hives are still common in the villages of most Asian countries (Akranakul 1976; Hepburn and Radloff 2011). As a result, feral nests of *A. cerana* are less hunted by man than nests of dwarf and giant honeybees (Akranakul 1976; Maa 1953; Otis 1990; Targari 1971; Wongsiri et al. 1996b; Wu and Kuang 1987). The several combs in an *A. cerana* colony are built parallel to each other, and have a uniform distance known as the “bee space” between them (Fig. 1.3). The body size of *A. cerana* workers is relatively small and there are two sizes of brood comb cells: smaller for worker and larger for drone brood. Queen cells are built on the lower edge of the comb. As in the other *Apis* species, honey is stored in the upper part of the combs, but also found in the outer combs, adjacent to the hive walls (Akranakul 1976; Maa 1953; Otis 1990; Wongsiri et al. 1996b).

1.4.1.1 The Distribution of *A. cerana*

A. cerana is the Asiatic honeybee or the oriental honeybee because they are only found in Asia, from Iran in the east to Pakistan in the west, and from Japan in the



Fig. 1.3 Combs of *A. cerana*

north to the Philippines in the south. Thus, *A. cerana* does not live only in tropical and subtropical areas of Asia, but also in colder areas as Siberia, northern China, and the high mountain area of the Himalayan region (Koeniger 1976b). *A. cerana* occurs at altitudes from 0 to 3333 m above the sea level (Rahman 1945). For many social insects, a tendency has been found that the higher the altitude and latitude the bees inhabit, the bigger the body size and the colony population is (Paspari and Vargo 1995). This relationship is the rule for *A. mellifera* (Ruttner 1988; Ruttner 2000; Hepburn et al. 2000), *A. cerana* (Verma et al. 1994a), *A. florea* (Ruttner et al. 1995), and for some stingless bee species (Pereboom and Biesmeijer 2003). A high degree of variation in size and coloration probably reflect the ecological diversity of *A. cerana*. The influence of latitude and altitude on the size of worker bees was also found for *A. cerana* in Vietnam (Niem et al. 1992). *A. cerana* colonies occur in all provinces of Vietnam (except Uminh forest) but their natural types are commonly found in mountain-forested areas such as Viet Bac, Hoang Lien Son, Truong Son; in coconut-growing provinces such as Ben Tre, Tien Giang, etc., and island districts such as Cat Ba, Phu Quoc, and Con Dao (Chinh et al. 1996).

1.4.1.2 Variation in the Indian Hive Bee

Like the other hive bee, *A. mellifera* Linnaeus, *A. cerana* too shows an enormous variation in body size, color, tongue length, foraging range, swarming tendency, and production capacity. In spite of this variation and its rearing for several decades, the species remains incompletely studied and its potential unexplored even today. Comprehensive studies on the biometry and taxonomy of *A. cerana* in India were undertaken by Kshirsagar (1976, 1983). Using over 60 biometric features of samples of 22 populations of this species from locations all over India, he differentiated seven ecotypes. Kshirsagar and Ranade (1981) showed that the bees in the southern-most

region along the coast, are the smallest, and the size increases northwards, the largest bee being found in the Kashmir Valley. A similar increase in size of the European bee towards northern latitudes has been reported (Ruttner 1988). Besides latitude, bee size and other morphological features show variation according to the altitude. Bees in Tamil Nadu plains are thus smaller than the bees in the Nilgiri hills, Tamil Nadu with an altitude of about 2000 m. Considering the correlation between the body size and biology of the bee with the natural vegetation as also the distribution of natural vegetation types in the country, the seven ecotypes identified by Kshirsagar (1983) are redefined and are listed in Table 1.1

Ruttner (1986, 1988) classified the different Asian hive bee populations into four groups, which he called as races of *A. cerana*, namely, *A. c. cerana*, *A. c. himalaya*, *A. c. indica*, and *A. c. japonica*. The former three races occur in India. Their distribution is as follows.

<i>A. c. cerana</i>	North-western region including Himachal Pradesh and Jammu and Kashmir
<i>A. c. himalaya</i>	North-eastern region including the north-eastern Himalayan states
<i>A. c. indica</i>	South India, including Kerala, Tamilnadu, Karnataka and southern Andhra Pradesh

Verma and coworkers made detailed investigations on the biometry and taxonomy of Indian hive bee from 50 localities, particularly in north India. According to these studies (Verma 1992), the three Indian races can be further differentiated into seven subgroups or ecotypes, two in *A. c. cerana*, three in *A. c. himalaya*, and two in *A. c. indica*. Intraspecific classification of the Indian *A. cerana* into seven ecotypes indicated by Kshirsagar (1983), and redefined here, seems to be well-founded. It is possible that by further detailed investigations, additional ecotypes and races can be found.

Deodikar et al. (1958) proposed that *A. cerana* originated by hybridization of a species of *Trigona* that had vertical multiple combs, with diploid species of *Apis*. They felt that the Indian hive bee possibly arose during Pleistocene glaciation, spread northwards through the Himalayas and gradually differentiated into the European hive bee. The center of origin of the *Apis* is considered to be the Indo-Malayan region. Because of this, there is a possibility of occurrence of several races, and varieties of *A. cerana* in this region.

Over the last two decades, great strides have been made following Ruttner’s (1988) first multivariate analysis of this species. Historically, unraveling the structural complexity of *A. cerana* (Fabricius 1793) has been a continuous process, the details of which were recently given by Radloff et al. (2010). They reported the first multivariate morphometric analysis of *A. cerana* across its full geographical range and identified the statistically definable morphoclusters and subcluster populations within them. Principal component (PC) plots, using both the first and second PC scores and the first and third PC scores, did not reveal distinct morphoclusters. However, a substructuring of the PC plots was obtained by introducing local labeling and running a hierarchical cluster analysis, using the mean scores for PC 1 to 3 to identify homogeneous morphoclusters. This approach revealed six main morphoclusters, which were defined (Radloff et al. 2010) as follows:

Table 1.1 Ecotypes of *A. cerana* F. in India. (Source: Kshirsagar 1983)

Geographic region	Latitude	Altitude (m)	Location of sample collection	Remarks
Kashmir valley	34°05'	1,586	Srinagar, Jammu and Kashmir	Largest ecotype in the country
Western Himalayas	31°43'	761	Mandi, Himachal Pradesh	Possibly includes the next two variants
Western Sub-Himalayas	30°05'	700	Kangra, Himachal Pradesh	Possibly variant of western Himalayas
Western Sub-Himalayan foothills	30°10'	630	Ranipokhari, Uttar Pradesh	Possibly variant of western Himalayas, and not ecotype
Eastern Himalayas	26°53'	1,500	Kurseong, West Bengal	Verma (1992) proposes three races in this region
Indo-Gangetic Plains and Aravali Hills	29°13'	440	Haldwani, Uttar Pradesh	Mahabaleshwar included due to its high altitude
	26°06'	53	Muzaffarpur, Bihar	
	26°05'	54	Guwahati, Assam	
	24°36'	1,195	Mount Abu, Rajasthan	
	17°56'	1,382	Mahabaleshwar, Maharashtra	
Central Peninsula	20°48'	27	Cuttack, Orissa	
	17°50'	767	Lammasingi, Andhra Pradesh	
	17°00'	670	Petlond, Maharashtra	
Western and eastern Ghats	15°20'	700	Castle Rock, Karnataka	Kodaikanal included due to its high altitude
	14°57'	700	Yellapur, Karnataka	
	12°57'	650	Sakleshpur, Karnataka	
	10°14'	2,343	Kodaikanal, Tamil Nadu	
Western and eastern Peninsular Coastal strips	14°25'	0	Kumtha, Karnataka	Smallest ecotype in the country
	11°55'	0	Pondichery, Pondichery	
	10°46'	97	Palghat, Kerala	
	08°44'	51	Tirunelveli, Tamil Nadu	
	08°05'	37	Kanyakumari, Tamil Nadu	

1. Morphocluster I: “Northern cerana,” which extends from northern Afghanistan and Pakistan through northwest India, across southern Tibet, northern Myanmar, China and northeasterly into Korea, far eastern Russia and Japan. Six subclusters or populations are morphometrically discernible within this morphocluster: (a) an “Indus” group in Afghanistan, Pakistan, and Kashmir; (b) a “Himachali” group in Himachal Pradesh, India; (c) an “Aba” group in Gansu and Sichuan provinces in China, northern China, and Russia; (d) a subcluster in central and eastern China; (e) a “southern cerana” subcluster in southern Yunnan, Guangdong, Guangxi, and Hainan in China, and (f) a “*japonica*” group in Japan and Korea.
2. Morphocluster II: “Himalayan cerana,” which includes the bees of northern India and some of southern Tibet and Nepal. Two subclusters are discernible within this morphocluster: the bees of the northwest, which are termed the “Hills” group, and those of the northeast, termed the “Ganges” group.
3. Morphocluster III: “Indian plains cerana,” which occurs across the plains of central and southern India and Sri Lanka as a fairly uniform population, long known as “plains cerana” in this subcontinent.
4. Morphocluster IV: “Indo-Chinese cerana,” which forms a compact group in Myanmar, northern Thailand, Laos, Cambodia, and southern Vietnam.
5. Morphocluster V: “Philippine cerana,” which is restricted to the Philippines, but with the exclusion of most of Palawan Island, which instead groups with morphocluster VI. Within these islands, there are subclusters, and these bees are termed after the major island groups located there: “Luzon,” “Mindanao,” and “Visayas” bees. The latter two subclusters show closer morphometric similarity than the former.
6. Morphocluster VI: “Indo-Malayan cerana,” which extends from southern Thailand, through Malaysia and Indonesia. This large area consists of a rather morphometrically uniform bee, below the south China Sea. Three subclusters are discernible within this morphocluster: (a) Palawan (Philippines) and Borneo bees; (b) Malay Peninsula, Sumatera, and some Sulawesi bees; and (c) Indonesia (Java, Bali, Irian Jaya, some Sulawesi, and Sumatera) bees. We must now consider how these results relate to earlier geographically large-scale analyses. When all the mesoscale morphoclusters of Radloff et al. (2010) are compared with the new macroscale results, the only discrepancies are that, in the former: (a) the bees of the Philippines were included with those of Indonesia and Borneo and (b) the bees of Japan are now placed in the northern Asia morphocluster of the latter. However, there are differences between the mapped morphocluster results of Ruttner (1988) and Damus and Otis (1997) and those of Radloff et al. (2010). These discrepancies are best explained by the sampling differences in each study, which affected the degree of morphometric discrimination of the honeybees of Japan. Ruttner (1988) had access to only a very small sample of large *A. cerana* from China and none from Russia. The only morphocluster I bees available to him were from the far northwest of the *A. cerana* range (Afghanistan and Pakistan) and some 6,000 km distant from Japan—the bees of which form a subcluster in a continuum of *A. cerana* morphocluster I. Gaps in the sampling inevitably resulted in the differences between Afghani and Japanese *A. cerana* being artefactually

magnified. The dataset of Damus and Otis (1997) was based on the much smaller bees of the more southerly oceanic islands (Philippines, Indonesia, Borneo, etc.) with the same effect.

1.4.1.3 Races of *A. cerana*

There are many different races of *A. cerana* as could be expected from the wide range of habitats it occupies. Bees of some of the races are the same size as some *A. mellifera*. However, *A. cerana* varies in size throughout its range, and tropical races are much smaller, with smaller colonies. Subspecies following Engel (1999) are as given below:

- A. c. cerana* (= “sinensis”)—Afghanistan, Pakistan, north India, China, and north Vietnam
- A. c. heimifeng*
- A. c. indica*—South India, Sri Lanka, Bangladesh, Burma, Malaysia, Indonesia, and the Philippines
- A. c. japonica*—Japan
- A. c. javana*
- A. c. johni*
- A. c. nuluensis*
- A. c. skorikovi* (= “himalaya”)—Central and east Himalayan mountains (Ruttner 1987) running. Native to Asia between Afghanistan and Japan, and from Russia and China in the north to southern Indonesia. Recently introduced to Papua New Guinea.
- A. cerana* builds a nest consisting of a series of parallel combs, similar to *A. mellifera*, and builds its nest within a cavity.

1.4.1.4 Studies on the Taxonomy of *A. cerana* in the World

A. mellifera is the best studied species in honeybees in particular and in social bees in general. Twenty-six subspecies and ecotypes are discriminated and have been studied in detail. Compared to *A. mellifera* there is very little research on the morphology of *A. cerana* (Verma 1990). Based on the analysis of 34 morphological criteria of 68 samples collected from different areas of Asia, Ruttner (1988) divided *A. cerana* into four subspecies.

A. c. indica

This is the subspecies with the smallest body size. It lives in the south of India, the south of Thailand, Cambodia, Vietnam, Malaysia, Indonesia, and The Philippines. The length of proboscis and forewing is 4.58–4.78 and 7.42–7.78 mm, respectively (Ruttner 1988).

A. c. cerana

This subspecies with the biggest body size of *A. cerana* occurs in northern parts of China, the northwest of India, the north of Pakistan and Afghanistan, and in the north of Vietnam. On average, the proboscis and forewing length measure 5.25 and 8.63 mm, respectively.

A. c. himalaya

The body size of this subspecies is intermediate between *A. c. cerana* and *A. c. indica*. It occurs in the east of the Himalayas from Nepal to northern Thailand. On average, the proboscis and forewing length measure 5.14 and 8.03 mm, respectively.

A. c. japonica

This subspecies is endemic in Japanese temperate climates except the island of Hokkaido. This subspecies is divided into two separate ecotypes: Honshi and Tsushima. The body size of *A. c. japonica* is relatively big, with an average proboscis length of 5.18 mm and an average forewing length of 8.69 mm. *A. c. japonica* gradually has been replaced by introduced *A. mellifera* (Okada 1986). The intraspecific classification of the Asiatic honeybee species, *A. cerana* is in a state of flux and uncertainty (Hepburn et al. 2001). Next to the four subspecies distinguished by Ruttner (Ruttner 1988), four other subspecies have been proposed: *A. c. abaensis*, *A. c. philippina*, *A. c. skorikovi* and *A. c. hainanensis* (reviewed by Hepburn et al. 2001a). Five of these subspecies occur in China: *A. c. indica*, *A. c. cerana*, *A. c. skorikovi*, *A. c. hainanensis*, and *A. c. abaensis*. *A. c. cerana* is divided further into five ecotypes known as Quangdong-Quangxi, Hainan, Yunnan north, and Changbei-Shan (Zhen-Ming et al. 1992). Based on multivariate morphometric analyses of 557 colonies of *A. cerana* from all the southern mainland of Asia, Hepburn et al. (2001b) have recently established that *A. cerana* is placed in three separable groups that are not entirely distinct morphoclusters of bees: (1) bees from the Hindu Kush, Kashmir, northern Myanmar, northern Vietnam, and southern China; (2) bees from northern India, Nepal, central Myanmar and Thailand, Cambodia, southern Vietnam, and southern China; and (3) bees from central and southern India, southern Myanmar, southern Thailand, and peninsular Malaysia. However, the nomenclature of these intraspecific taxa of *A. cerana* still remains unadjusted in this chapter. Deviations in the intraspecific classification of *A. cerana* probably reflect differences in sampling and methodology. *A. cerana* is the east Asiatic counterpart of *A. mellifera*. Its morphology and behavior are so similar to *A. mellifera* that for a long time it was considered as an *A. mellifera* subspecies (Buttel-Reepen 1906). However, it has several species-specific characters and is genetically separated from *A. mellifera* (Ruttner and Maul 1983). It is not true that *A. cerana* is smaller than *A. mellifera*. These species overlap in size. The northern types are generally larger than southern types. Ecological requirements of *A. cerana* are about the same as those of *A. mellifera*.

This species also succeeded in colonizing forested areas in the cool temperate zone (northern China to Ussuria in East Siberia). There are four subspecies reported for *A. cerana* namely, *A. c. cerana* in Afghanistan, Pakistan, north India, China, and north Vietnam, *A. c. indica* in south India, Sri Lanka, Bangladesh, Burma, Malaysia, Indonesia, and the Philippines, *A. c. japonica* in Japan and *A. c. himalaya* in Central and east Himalayan mountains (Ruttner 1987). Thus, its area of distribution is very large; it extends from west Afghanistan to Japan.

Genetic variance in morphological characters of *A. cerana* subspecies in the Himalayan region have been identified. These subspecies are named *A. c. cerana*, *A. c. himalaya*, and *A. c. indica*. Each subspecies has further locally adapted populations called ecotypes, which differ from each other in several biological and economic characters. For example, three ecotypes of subspecies *A. c. himalaya* that correspond to geographic distribution in: (1) the Naga and Milo Hills, (2) Brahmaputra Valley and Khasi Hills, and (3) the foothills of the north-east Himalayas have been identified. In some parts of the Hindu Kush Himalaya, *A. c. cerana* matches the European hive bee *A. mellifera* in commercial value and has spectacular potential for further genetic improvement.

When kept sympatrically, *A. cerana* and *A. mellifera* colonies frequently rob each other (Koeniger 1982). In Japan, *A. cerana* (originally the only honeybee) is now replaced by imported *A. mellifera* colonies. Another cause of failing coexistence of the two species is attempted intermating, which produces lethal offspring (Ruttner and Maul 1983). Another problem is shifting of parasites from one species to the other as the geographical isolation is broken by humans. *Varroa* mite, which is coadapted to *A. cerana* and is parasitic on the drone brood of this species causing no serious problem has shifted to the unadapted *A. mellifera* and is a serious pest on it. *A. cerana* colonies are smaller than that of *A. mellifera* and so are the honey yields.

The eastern cavity-nesting honeybee, *A. cerana* F., is widespread over Asia and occupies a distribution extending from Afghanistan to China and from Japan to southern Indonesia (Ruttner 1988). This species has been grouped based on morphometric analyses (Ruttner 1988) in four subspecies with different distribution ranges: *A. c. cerana* from Afghanistan, Pakistan, north India, China and north Vietnam, *A. c. indica* from south India, Sri Lanka, Bangladesh, Burma, Malaysia, Indonesia and the Philippines, *A. c. japonica* from Japan and *A. c. himalaya* from central and east Himalayan Mountains (Smith 1991). These subspecies include many populations some of which are geographically isolated, such as those in the Philippines archipelago.

It is suggested that *A. indica* originated from primitive diploid by: (1) polyploidy combined with adaptive mutations for multiple enclosed comb habits or (2) hybridization at diploid level among closely related *Apis* and *Trigona* followed by polyploidy during pleistocene glaciation in the Indo-Malayan region. Primitive *A. indica* gradually evolved into *A. mellifera*, which differentiated further into a number of African, Eurasian, and Sino-Japanese races during the course of the passage through the three main northern migratory routes of the Himalayas. Bearings of these observations on the practical problems of breeding better strains of Indian bees are discussed.

As with *A. mellifera*, *A. cerana* occurs over a huge geographical area, and it varies in size throughout its range: tropical races are smaller bees, with smaller colonies. *A. cerana* bees are smaller than *A. mellifera*, and they form colonies smaller than

A. mellifera. The foraging range may be also smaller. There are many different races of *A. cerana*, as could be expected from the wide range of habitats it occupies from temperate mountain regions to tropical islands. It is possible that some races of *A. cerana* will in the near future be recognized as separate species. Other honeybee species in Asia showing behavior similar to *A. cerana* are *A. koschevnikovi*, *A. nigrocincta*, and *A. nuluensis*.

A. nigrocincta

A. nigrocincta Frederick Smith, 1861 is a species of honeybee that inhabits the Philippine islands of Mindanao and Sangihe as well as the Indonesian island of Celebes or Sulawesi. The species builds nests in cavities like the closely related *A. cerana*. In fact, there are few substantial differences between the two species: the genitals of the respective drones, for instance, are identical. However, there are small morphological differences, genetic polymorphism in the mitochondrial DNA, as well as behavioral differences. In areas where the *A. cerana* and *A. nigrocincta* live together, they can most immediately be distinguished by their coloration and size: *A. cerana* tends to be darker and smaller while *A. nigrocincta* tends to be larger and has a yellowish clypeus (the lower area of the face). The architecture of the colonies is also a point of difference: the opening of the drone cell of *A. cerana* is covered in wax, under which there is a conical cocoon with a central hole or pore. In *A. nigrocincta*, however, the cell of the drone has a narrow opening, without a hard wax cap and hole. In addition, the queens of *A. nigrocincta* generally create colonies with greater numbers of drones than those of *A. cerana*. Another noticeable behavioral difference between the species is the time of day at which they prefer to gather pollen. *A. nigrocincta* contracts the parasite-caused honeybee disease Varroaosis by playing host to the species of *Varroa* mite known as *Varroa underwoodi*. In this way, they are similar to *A. c. nuluensis*, which is also susceptible to the same species of parasite.

A. c. nuluensis

A. c. nuluensis Tingek, Koeniger & Koeniger, 1996 is a subspecies of honeybee described in 1996 by Tingek, Koeniger & Koeniger. The geographic distribution of the subspecies is the southeast Asian island of Borneo, politically divided between Indonesia, Malaysia, and Brunei. *A. c. nuluensis* is one of a number of Asiatic honeybees, including the more obscure *A. koschevnikovi* and *A. nigrocincta* (the latter of which has nearby habitat on nearby Sulawesi and Mindanao islands). While this was originally described as a species, it has since been determined to represent a geographic race (subspecies) of the widespread *A. cerana* (Engel 1999). Like many honeybees, *A. c. nuluensis* is liable to infestation by the parasitic *Varroa* mite, although in this case the particular species is *V. underwoodi*. (In this aspect, *A. c. nuluensis* is similar to *A. nigrocincta*).

A. koschevnikovi

Koschevnikov's Bee, or *A. koschevnikovi*, Buttel-Reepen, 1906 is a species of honeybee which inhabits Sabah, Malaysian and Indonesian Borneo, where it lives conspecifically with other honeybee species such as *A. cerana* (specifically *A. c. nuluensis*). The individual bees are slightly larger than *A. cerana* found in the same locality, but otherwise the colonies are similar in size and construction. They are also known as red bees due to a reddish hue when clustering. This species was named for a short period as *A. vechti*. The species was first described by Buttel-Reepen, who dedicated it to Koschevnikov, a nineteenth-century pioneer of honeybee morphology. The species was described again by Maa in 1953, this time with the name *A. vechti*. It was finally rediscovered by Tingek et al. in 1988. *A. koschevnikovi* hosts a unique species of the honeybee parasite *Varroa*, named *Varroa rindereri*. (Guzmán et al. 1996) Although this parasite species is quite similar to *V. jacobsoni*, it is perfectly differentiable. It has only been reported in colonies of *A. koschevnikovi* in Borneo and seems to be specific to that species, as it has yet to be observed crossing over to colonies of *A. cerana*, even when they live in the same apiary. These gentle species of bees have long been managed as useful honeybees in many parts of Asia and their honey and wax valued.

A. cerana does not produce propolis. Since it is a cavity nesting bee, it is possible to keep *A. cerana* in a wide range of hive types and movable-frame hives and movable-comb hives (top-bar hives) have been developed for *A. cerana* and the other cavity nesting hive bees. Their gentle nature allows these bees to be kept close to home and in many places are kept in logs hanging from the eaves of houses, or in specially designed cavities built into the walls of houses.

A. cerana, the Asian hive bee, is particularly important to mountain farmers as a source of cash income. This species is well-suited both to the climatic conditions in the region and to the farming practices that are typical of these marginal, mountainous areas. It has the ideal characteristics to ensure the pollination of mountain crops, having adapted its foraging patterns to suit the changing flowering and nectar production rhythms that result from the uncertain and variable climatic conditions in mountain areas. It can work under cool conditions up to an altitude of 3,000 m and is ideally suited as a pollinator of early-flowering crops like almonds, peaches, and plums. Kept in hives in the backyards, these bees pollinate kitchen garden crops, usually the main source of vegetables. The indigenous bee offers a further advantage in that it keeps going even under adverse conditions; if the situation becomes really difficult, the colonies may migrate temporarily but the bees come back to their hives when conditions allow them to do so.

1.5 Characteristics of the Indian Hive Bee

The nest consists of several parallel combs with a uniform distance between them. The nests have usually six to eight combs. A wide variation occurs in the number depending upon the period of stay of the nest in the location, space available in the nest

site, and its shape. Sometimes only three to four combs, which are narrow but about a meter long are found. In natural nests that lived for over 2 years, up to 15 normal-sized combs were found that yielded over 10 kg of honey. Individual combs are uniform in thickness, unlike the combs of the dwarf or rockbee. The combs usually have honey stores in the upper part, brood in the middle and lower parts, and pollen stores on the sides of the comb, adjacent to the brood. Drone cells are constructed along the lower part of the comb. These are conspicuously larger, and have raised covers with a pore in their middle. Queen cells under normal conditions are built along the lower edge. All the combs have similar functional differentiation, but usually the central combs in the nest contain brood while the outer combs have little or no brood, the entire area being utilized for honey or pollen stores. Combs are about 25 mm thick. The brood cells are about 11.5 mm deep, and vary in width usually from 4.17 to 4.83 mm, the size increasing towards north (Muttoo 1956; Deodikar et al. 1958; Singh 1983). Bees in the Kashmir valley may have over 4.9 mm wide cells, being at the same latitude as Peshawar, where the size is 4.87 mm (Ruttner 1988).

The egg stage in all castes lasts 3 days, the larval stage of workers 4–5 days, drones 7 days, and queens 5 days; the pupal stage is 11–12 days for workers, 13–14 days for drones, and 7–8 days for the queen. The total development is completed in 18–19 days by the worker, in 24 days by the drone, and in 15–16 days by the queen (Muttoo 1956). There is some variation in these periods depending on the type of the bee in different parts of the country. Shah and Shah (1982) made a comparison of the Kashmir strain with other hill type in India along with the European bee. A brief account of this is given in Table 1.1 as it also indicates the general behavior and performance limits of the Indian hive bee.

The Indian bees are considered as mild and are easy to handle. Its sting releases half the amount of alarm pheromone as does the sting of the European bee (Morse et al. 1967). Beekeepers often handle the colonies bare handed without smoking them, (Abrol 1997). Perhaps this is one of the reasons for repeated handling of the colonies even by the inexperienced. This is a cause of disturbance and colonies desert. The bees have, like other Indian bee species, quite effective defense mechanisms against the usual enemies and predators. They exhibit shimmering behavior, but since the nest is in the dark, only the sharp hissing sound is indicative of this behavior. A knock on the hive elicits this behavior and a 0.5 s long, clearly audible hissing results. Group defense is another strategy in which about 30 bees form a group at the entrance with the tip of their abdomen raised, on perceiving danger from an attacking hornet. Perhaps they release Nasanov pheromone to elicit the group behavior. This along with sharp repeated hissing sounds from the nest makes the hornet abandon its attempt to attack (Ruttner 1988). The Indian bee is often blamed for its desertion or absconding tendency. Bees leave the nest when disturbed or when faced with inimical atmospheric conditions, or during periods of food scarcity. In the tropics, bees are exposed to attacks of a wide range of predators and enemies. Common among them are wasps, hornets, and even ants that can really be constant irritants. Absconding behavior is not peculiar to the Indian bee; it is found equally characteristically in the tropical African and Middle Eastern races of the European hive bee. Absconding is largely a problem of management than of bee behavior. The problem can easily be

solved by understanding the reasons for absconding and removing them before the colony attempts to desert.

The Indian hive bee does not use propolis as the European bees do. This may be an adaptation to tropical climate, where hive ventilation assumes importance. The cracks in the floor board or gaps in the hive walls or frame joints are not sealed. This may attract pests like wax moth. One of the characteristic features of the hive bee is fanning used for ventilation of the hive. During nectar flows, large quantities of water have to be removed from the dilute honey in combs and ripen it. The moisture-ridden air has to be removed from the hive. For this purpose, bees undertake fanning vigorously, and it is most visible at the hive entrance. The Indian hive bee fans with its head facing away from the entrance. Contrastingly, the European bee fans with its head towards the entrance. In their experiments on introducing queens of the European bee into the colonies of the Indian bee, Dhaliwal and Atwal (1970) observed the workers of both the species fanning side by side, but heads oriented in opposite directions.

Honeys from the Indian hive bee have (Phadke 1967a) about 20 % water. The levulose content is about 36.5 %, dextrose about 33.4 %, while the nonreducing sugars are about 3.4 %. The European honeys have usually up to 17 % water, about 41 % levulose, and about 36 % as dextrose. The high water content in Indian honey can be attributed to the tropical humid climate prevailing in the major honey-producing areas. This seems to be confirmed from similar studies on honeys from Mahabaleshwar, Maharashtra with a subtropical climate (Phadke 1967b). Water content in samples of eight types of unifloral honeys from here was between 17.2 and 19.0 %. *A. cerana* honeys have a high invertase content and low diastase, catalase and glucose oxidase values, compared to those of *A. mellifera*. The average values found by Wakhle and Desai (1983) in 17 samples from all over India were 6.55 for diastase, 25.60 for invertase, and 1.44 for catalase. Heat and long storage under tropical conditions reduce the enzyme content.

Microscopical analysis of apiary honeys (Seethalakshmi 1983) shows generally a high pollen content. The absolute pollen count of 12 samples studied varied from 26,000 to 225,000 grains per 10 g. The pollen types in the honeys showed a wide range. The wax of the Indian hive bee has a melting point of 65 °C and an acid value of 6.54 (Phadke et al. 1969). It resembles the rockbee wax in having a low acid value compared to that of the European beeswax. Analyzing the factors causing the low acid value, Phadke et al. (1971) state that the Indian beeswaxes have only about 8 % as hydrocarbons. This is nearly half that present in the European waxes. Consequently, the total alcohol content is more in the Indian waxes. In all other respects, the Indian beeswax is similar to the European wax. It can therefore replace imported beeswax in pharmaceutical and cosmetic industries.

1.5.1 Special Characteristics of *A. cerana*

A. cerana is still found in the wild in many areas. Nests are built mostly in forests and areas of scrub in tree holes, fallen logs, crevices and others, and at altitudes of

up to 3,000 m. Unlike most other wild bees, however, *A. cerana* can be “domesticated.” Colonies can be caught in the wild and maintained in simple log or wall hives with a minimum of inputs. Also, the swarms can be multiplied and maintained over generations. These bees have adapted to the diverse and often extreme climatic, biological, and agricultural conditions that exist at higher altitudes, with the development of subspecies suited to specific agroclimatic zones. They can survive low winter temperatures, extreme fluctuations of temperature, and long periods of rainfall, and continue to fly on dull days and during rain showers. They feed on a range of flowers. The bees have developed a capacity to fight, escape, and adjust to local parasites, diseases, and other enemies. They are resistant to the effects of mites like *Varroa* spp., which have virtually crippled the beekeeping industry in some parts of the western hemisphere.

1.6 *A. cerana* and Mountain Farmers

Farmers in mountain areas especially the Himalayas, profit from *A. cerana* in a number of ways. First, honey and other bee products are a source of cash income, nutrition, and medicine. Poor and landless farmers in remote areas find bee colonies in the forest, protect them, and harvest small amounts of honey from them. Local tradition allows them to claim “ownership” of such colonies whilst leaving them at their original nesting site, thus acting as “guardians of biodiversity.” Besides conserving the bees, this practice of hunting helps very poor and marginalized people to make a part of their livelihoods. Farmers with more space and resources keep bees in simple homemade log or wall hives kept close to the house. Over centuries, farmers have further selected these local bees for useful characteristics. Many different strains have been identified some of which, for example, show higher honey production or more passivity. Once bees are kept in hives, it is easier to collect both honey and other products. Second, farmers benefit from the bees as pollinators. *A. cerana* has the ideal characteristics to ensure pollination of mountain crops. It has adapted its foraging patterns to suit the changing flowering and nectar production rhythms that result from the uncertain and variable climatic conditions in mountain areas. It can work under cool conditions and is ideally suited as a pollinator of early-flowering crops like almonds, peaches, and plums. Kept in backyards, the bees pollinate kitchen garden crops, usually the major source of vegetables. The indigenous bee offers a further advantage in that it keeps going even under adverse conditions; if the situation becomes really difficult, the colonies may abscond temporarily but the bees reoccupy their hives when conditions allow them to do so. *A. cerana*, and other bees, play an often unrecognized role in combating soil degradation by enhancing the replenishment cycle: more wild plants are pollinated, more seed is produced, more young plants grow, and more biomass is available to return to the soil. Mountain communities have adapted themselves to the harsh realities of life in these marginal areas. They have evolved a pool of indigenous knowledge that enables them to survive the extremes and the variability. The strategies are based

on a broad risk coverage against famines, drought, and other natural disasters, and they include diversification and integrated use of separate elements like crops, livestock, fruits, vegetables, forests, and honeybees, that together ensure self-sufficiency. Beekeeping with *A. cerana* is an integral part of this indigenous approach and the indigenous knowledge that has evolved is a part of the natural heritage of these people.

1.7 Declining Populations of *A. cerana*

A. cerana is being threatened by several factors, which include changes in land use and intensification of agriculture, which are leading to a loss of wild habitat, reduction in species availability for feeding, and death from pesticides; and on the other, introduction of *A. mellifera* into indigenous areas of *A. cerana* is encouraging farmers to replace *A. cerana* with the exotic bee, and introducing diseases and parasites against which *A. cerana* has no resistance. The exotic bee species *A. mellifera* have proved much superior and less problematic than the indigenous honeybee *A. cerana* (Atwal 2000). It has been found to yield several times more honey than *A. cerana* besides having many other useful traits such as resistance to diseases, low swarming tendency, and gentle temperament.

The introduction of *A. mellifera* to Japan reduced the local *A. cerana* population to the point where “*A. cerana* is found only in remote mountainous areas, almost like a relic, which could soon become an endangered species” (Ruttner 1988). Similar situation occurred in India and other Asian countries.

It is expected that *A. mellifera* will serve the commercial beekeeping in most parts of the state, whereas *A. cerana* will continue to subsist in marginal beekeeping areas, which are less suitable for *A. mellifera*. The major factor for decline in populations of *A. cerana* has been the nonacceptability of this species by beekeepers and the farming community in comparison to *A. mellifera*. Pesticide use is a particular problem in areas with cash crops—crops that can most benefit from *A. cerana*. In some parts of the mountainous areas, formal policy is leading to systematic destruction of wild habitat. China, for example, made a policy decision to transform wild mountain territories into fruit orchards, designated “economic forests,” useful insect populations have been reduced to almost nil, and farmers have had to resort to pollinating apple trees by hand. Introduction of disease through exotic species is a widespread problem. The four indigenous honeybee species (*A. cerana*, *A. laboriosa*, *A. dorsata*, *A. florea*) have coexisted for centuries without transfer of diseases and parasites. When *A. mellifera* was introduced, it brought with it European Foulbrood (EFB) and Acarine disease. In some areas, *A. cerana* was close to extinction, but resistant populations are now emerging. Other bee species have also been affected. The *A. mellifera* parasite *Melisococcus plutonius* has been detected in colonies of *A. laboriosa*, for example. There has also been transfer in the opposite direction: the *A. dorsata* parasite *Tropilaelaps clareae* transferred to *A. mellifera* colonies, and was spread by them.

There are very few areas in the world where indigenous species of honeybees other than *A. mellifera* still exist, and even fewer with indigenous honeybees that can be kept in hives and managed by farmers. Over centuries, farmers in remote and inaccessible areas of the Himalayan region have developed techniques for keeping and benefiting from the indigenous bee *A. cerana*. These bees can be maintained in hives using simple resources at altitudes of up to 3,000 m. They have adapted to the harsh conditions of the high mountain areas and evolved the characteristics necessary for survival. Unlike exotic introductions, *A. cerana* is well-suited both to the climatic conditions in the region and to the farming practices that are typical of these marginal areas. With its ability to fly and pollinate early in the season and under rainy conditions, *A. cerana* is poised to play a significant role in supporting the move towards growing of cash crops in these upland areas. But, at the same time, its survival is threatened unwittingly both by changes in farming practices—with loss of native habitats and increased use of pesticides—and by well-meant development interventions supporting the introduction of *A. mellifera*. The threat to *A. cerana* is to a great extent the result of lack of knowledge—about the bee itself, about the role it plays within farming systems, about the role it plays in maintaining biodiversity, and about the impact on it of interventions. Many people see bees simply as producers of honey and wax. Although this is a very important aspect, their major role as pollinators tends to be overlooked. Indigenous bees are needed as part of the natural cycle of maintaining wild indigenous plant species—particularly the rare endemic species that represent an important element of the local biodiversity. At the same time, bees play an important role as pollinators in farming, ensuring pollination and increasing productivity. As cash crops become more important, so too do the insects needed to ensure that pollination is as effective as possible.

1.8 Problems with *A. cerana* Beekeeping

It has been claimed that *A. cerana* (also known as Eastern honeybee, Asiatic hive bee, Mee Bee) is the exact equivalent, in the eastern part of the Old World, of its occidental sister species *A. mellifera*. “It has an equally wide area of distribution with a similar capacity for a broad spectrum of adaptations” (Ruttner 1988). However, although a honey producer, it does not hoard large quantities of honey and it is therefore not the preferred species for honey production, compared with *A. mellifera*.

1.8.1 Description of *A. cerana*

On average, *A. cerana* is smaller than *A. mellifera*. *A. cerana* is the third smallest of the nine species of honeybee (Koeniger et al. 2010). However, it should be noted that there are some genotypes of *A. mellifera* that are smaller than medium-sized *A. cerana* genotypes and there is no significant difference between the smallest genotype of both species (Ruttner 1988). However, the bigger *A. mellifera* genotypes considerably outsize even the largest *A. cerana* genotypes. *A. cerana* has distinctive stripes on its body compared to its European cousin *A. mellifera*.

1.8.2 Competition and Floral Resources

There is relatively little written about the floral preferences of *A. cerana*. In 1958, Miyamoto noted that *A. cerana* in Japan visited a wider variety of plant species, including natives, compared to the limited floral preferences of *A. mellifera* (Ruttner 1988). In a 1968 German study of honey from *A. cerana* and *A. mellifera*, clear differences in the pollen spectrum were collected by the two species despite them operating at the same time and in the same vicinity (cited in Ruttner 1988). Some studies claim that *A. mellifera* is clearly dominant over *A. cerana* in common feeding locations (Sakagami 1959; Dhaliwal and Atwal 1970; Ruttner 1988). Another, more recent study suggests the opposite. In the Solomon Islands, substantial losses of exotic *A. mellifera* honeybees were attributed to *A. cerana* robbing *A. mellifera* hives and increasing competition for floral resources (Anderson et al. 2010). More widely accepted is that *A. cerana* does well in disturbed or extensively modified habitats. For example, in Hong Kong, *A. cerana* visits 86 % of plant species and pollinators so successfully as to maintain that island's diverse flora (Corlett 2001; Oldroyd and Wongsiri 2006).

In one study of *A. mellifera* conducted in the Philippines, researchers found that the endemic bees, *A. cerana* and *A. dorsata*, negatively affected the growth of *A. mellifera* colonies in a forest ecosystem by aggression and robbing of stores. However, this finding was not duplicated for colonies studied in industrial or agricultural areas. Rather, the population growth of *A. mellifera* in an agro-ecosystem was significantly higher than in the industrial or forest environments. The abundance of melliferous plants in the agro-ecosystem enhanced the population build up of *A. mellifera*. They conclude that in spite of the diversity in a forest ecosystem, the exotic species *A. mellifera* failed to exploit the nectar and pollen sources of most plant species. This indicates that *A. mellifera* did not adapt to natural forest conditions in the tropics (Manila-fajardo and Cervancia 2003).

In nestmate recognition experiments, *A. cerana* was among the bee species that did not exhibit aggressive responses to the presence of other bees in their nests (Breed Deng et al. 2007). These authors suggest that robbing of stored food may be more characteristic of *A. mellifera* than other species in the genus *Apis*. Similar reports appear in Ruttner's (1988) account of this species. In relation to *A. mellifera* robbing efforts, he writes that "no effective defensive reactions are developed in *A. cerana*" (Ruttner 1988). Indeed, not only can intruders pass unimpeded but *A. cerana* bees inside a robbed colony were observed feeding the intruding *A. mellifera* robber bees.

1.8.3 Colony Size and Abundance

There is divergent evidence about the size of *A. cerana* colonies. A recent report of a full-sized colony described it as containing about 1,500 g bees, 700 g brood, about 4 kg of honey, and about 400 g of cells filled with pollen (Koeniger et al. 2010). Other reports cite usual colony sizes of 1,400–2,000 bees or between 10,000 to 20,000 bees

(Makhdzir and Osman 1980 cited in Ruttner 1988; Okada 1985 cited in Ruttner 1988). Colony size seems to depend on nest cavity availability (reported further). *A. cerana* tolerate a wide range of temperatures—from 5 °C to 45 °C; however, when compared to *A. mellifera* at 50 °C, *A. cerana* survived for a much shorter time while at 5 °C they equaled *A. mellifera* survival rates (Verma and Edwards 1971 cited in Ruttner 1988). There is evidence from Ussuria, Kashmir, Japan, and China that *A. cerana* are active at lower temperatures compared to *A. mellifera* and that they are therefore more active earlier in the morning than *A. mellifera* and can start flying earlier in spring than *A. mellifera* (Ruttner 1988). However, it should be noted that these data could be specific adaptations of certain ecotypes and may not be generalizable across whole species.

1.8.4 Defense Behaviors

An important cautionary note should be observed from the outset about the behavior of *A. cerana*. Most research has been done on dead bees with fewer descriptive studies of live specimens and very few experimental studies of comparative species characteristics. Therefore, it is not clear whether *A. cerana* defense behavior observed in the field at one location and point in time should be generalized to other times/places or for the species as a whole (Ruttner 1988).

Much has been written about colony defense behaviors and the consensus for *A. cerana* is that it is generally reported as being mild, tolerant, and timid (Ruttner 1988) in the context of attacks from European genotypes of *A. mellifera*. However, there are some unique behaviors associated with this species associated with colony defense: (a) abdomen shaking, (b) hissing (like a snake) in response to knocks or interference with the combs, (c) group defense via mob capture of large wasps near the nest entrance, and (d) stinging behavior. In Japan, Ono et al. (1995) noted one additional colony defense behavior in response to attack from a hornet: recognition and removal of a marauding wasp pheromone before it has a chance to attack other hornets. There is an important early paper by Sakagami, which outlines the competition and interaction of *A. mellifera* and *A. cerana* honeybee species in observations at mixed colonies (Sakagami 1959). In Japan, the endemic *A. cerana* species was gradually replaced by *A. mellifera* with records of *A. cerana* extinction dating back to 1925 since *A. mellifera* was first introduced into that country in 1876. Japanese (alongside many other) apiarists preferred the introduced *A. mellifera* species given the ease with which they adapted to movable frames and their greater honey production. Sakagami summarized that in general *A. cerana* is more tolerant and less aggressive than *A. mellifera*. According to him, with respect to interspecific conflict (in mixed colonies), *A. mellifera* usually took the dominant position in both aggressiveness and agility. He notes the superiority of (*A. mellifera*) species in terms of their larger colony size, strong fighting capacity, and protection afforded by humans. Another difference between the two species was noted in terms of their foraging behavior. Citing Hachinow 1954, Sakagami writes: (*mellifera*) have a tendency to

concentrates their effort on a major nectar source whilst (*cerana*) tend to forage from numerous minor sources. He also makes the point that under natural conditions, the species would interbreed very rarely, if at all.

Aligning with this assessment of less aggression of *A. cerana* compared to *A. mellifera* is evidence of their stinging behavior. Oldroyd and Wongsiri (2006) wrote that *A. cerana* are more likely to retreat inside the nest than to sting on the approach of a mammal. In one experiment, although *A. cerana* does sometimes attack an intruder (an artificial mouse made of felt)—afterwards no stings were detected in the felt—whereas *A. mellifera* stings were extensive on the same target. Moreover, the sting of an *A. cerana* worker bee contains about half the quantity of stinging material (isopentyl acetate) compared to those of *A. mellifera* worker bee stings. However, it has been reported that *A. cerana* stings have an effect for considerably longer than *A. mellifera* stings. Finally, *A. cerana* appears to have the least well-developed barbs on the sting lancet compared to all other *Apis* species barbs (Ruttner 1988).

1.8.5 *Flight Patterns and Swarming*

A. cerana bee flights are reported to be similar to fly flights in that they are rapid and unpredictable compared to *A. mellifera* flight patterns. There is also some unpublished evidence that *A. cerana* colonies in hives demonstrated 5.5 times as much flight activity relative to the number of bees in a colony compared to *A. mellifera* (Ruttner 1988). However, they tend not to fly far from their nests to forage; one source claims that this distance can be as far as 750 m but that 300 m is more typical (Punchihewa 1994). Swarming activity associated with *A. cerana* reproduction is reported differently in different countries. In Japan, Tokuda recorded one, two, or three swarms per colony per year, while researchers in Pakistan recorded an average of eight swarms per year (Ruttner 1988). Koeniger et al. (2010) report that in tropical conditions swarms can survive and travel for several weeks. However, longer periods of nectar scarcity or extended periods of rain will put the survival of a swarm at risk. In Taiwan, Fen Tsung Deh reported regular seasonal migration swarms by *A. cerana* between humid mountain areas and flatter areas (Ruttner 1988). In Australia, extensive field observation of the limited incursions to date indicate that there may be a difference in swarming behavior depending on whether they are: (a) reproductive swarms (1–2 per year) or (b) absconding swarms (up to 7 per year). There may also be a difference in swarming behavior depending on whether *A. cerana* is in colonization (“bunker down after moving in”) or invasion mode (“up stakes and spreading out”). Swarms of *A. cerana* who abscond (i.e., desert their nests) generally do so in response to a shortage in floral resources, an attack or approach by predator/s, or disease outbreak, e.g., from wax moths. Absconding behavior is reported differently in different countries—with more frequent reports of absconces from Thailand and in temperate Japan and less frequent reports in South Asia (Ruttner 1988).

1.8.6 Nesting

As a species of cavity dwelling bees, *A. cerana* colonies nest in hollow trees, caves, rock-clefts, walls roof spaces and rafters, as well as cavities provided by birds, small mammals, and tree dwelling reptile species. In Sri Lanka, it was thought that *A. cerana* require fully enclosed cavities to nest (Punchihewa 1994), however, this is not observed in other places. It is widely thought that *A. cerana* occupy smaller hives than *A. mellifera* given their smaller physical size and colony size. *A. cerana* occupy smaller hives than *A. mellifera* when they are farmed and there is evidence that *A. cerana* colonies fail in standard size hives (Pandey 1977; Ruttner 1988). However, in natural environments, *A. cerana* build nests in cavities with volumes as small as 4.5 L to as large as 97 L (Inoue et al. 1990 cited in Olroyd and Wongsiri 2006). In one study, *A. cerana* were found in a tree cavity with a diameter of 12 cm (Olroyd and Wongsiri 2006:153). Unlike other cavity nesting bees, cavity entrances of *A. cerana* nests vary widely (from 2 to 100 cm²) and are often found within 1–2 m off ground level; Inoue et al. 1990; Seeley et al. 1982; Olroyd and Wongsiri 2006). *A. cerana* are one of the cavities-nesting species, which thermoregulate nest temperatures. Where external ambient temperatures may vary between 12 and 36 °C, this bee species is able to maintain the brood nest temperature in the range of 33–35.5 °C. In particularly hot weather, *A. cerana* will use evaporative cooling mechanisms, collect water, and cluster outside the nest. In particularly cold weather, *A. cerana* has been observed using metabolic heat to warm brood nests. There is some evidence (well-documented in Ruttner 1988) of *A. cerana* routinely dismantling old combs in nests in order to build new cells upon it. Arguably, this may contribute to more hygienic practices at the comb site, but less as the old wax debris accumulates on the bottom of the hive and provides a suitable medium for wax moths (Ruttner 1988).

Species of *A. cerana*, *A. dorsata*, *A. laboriosa*, *A. florea*, and *A. adreniformis* are the primary host of three different genus of mites, *Tropilaelaps*, *Euvarroa*, and *Varroa*. Mites in the genus *Tropilaelaps* are parasites of the giant honeybees of Asia (*A. dorsata* and *A. laboriosa*; Anderson and Morgan 2007). Some are occasionally observed inside *A. cerana* colonies in Asia (Ruttner 1988; Otis and Kralj 2001). However, except for one rare instance in Asia (Anderson and Morgan 2007), there is no other evidence that these reproduce on *A. cerana* brood (Otis and Kralj 2001). Mites in the *Euvarroa* genus are hosted by *A. florea* and *Apis adreniformis*.

A. cerana is host to three different kinds of *Varroa* mites—including *V. jacobsoni*, *V. underwoodi*, and *V. destructor* depending on the genotype of *A. cerana* (Anderson and Trueman 2000). So far only genotypes of *A. cerana* from northeast mainland Asia and the Japan region carry the forms of *V. destructor*, which are so damaging to *A. mellifera* globally. The java genotype of *A. cerana* carries mites that have long been known to be harmless to *A. mellifera*. However, in 2008, a harmful form of the mite was detected in Papua New Guinea (Anderson 2008). This mite did not accompany the java genotype of *A. cerana* into the Solomon Islands (Anderson et al. 2010). The bee in the Solomon Islands carries a harmless form of the java genotype of *V. jacobsoni*. *A. cerana* can effectively remove *V. jacobsoni* through grooming

behavior consisting of self-cleaning, grooming dance, nestmate cleaning, and group cleaning. *A. cerana* worker bees can also rapidly and effectively remove *V. jacobsoni* mites from the brood. However, *A. mellifera* does not demonstrate such a high level of grooming behavior.

1.9 Stock Improvement

Many of the previously mentioned subspecies and ecotypes of *A. cerana* are at present not economically viable. Therefore, to achieve stock improvement, different *A. cerana* subspecies and ecotypes should be accumulated at a central location and superior genotypes be identified. Another important prerequisite for stock improvement is to evolve efficient queen rearing for *A. cerana* and also establish isolated mating stations for pure line breeding. The latter is essential because instrumental insemination in *A. cerana* has unexpectedly turned out to be a difficult task due to very low volumes of semen ejaculated by drones. During the course of evolution, *A. cerana* has developed certain behavioral characteristics such as frequent absconding and swarming, which are essential for the survival of colonies but undesirable from a beekeeping point of view.

1.10 The Advantages of Beekeeping with *A. cerana*

The advantages of beekeeping with *A. cerana* are as follows:

1. Colonies available in the wild.
2. Better adapted to the local climate.
3. More resistant to parasites and disease.
4. Less demanding in hive specifications.

Development interventions in mountain farming areas are generally focused on improving productivity, introducing cash crops, and developing other income-generating activities—all of which can be provided or supported by *A. cerana*. Furthermore, in remote and isolated areas, and where the cash economy is poorly developed, interventions—to be successful—must be based on a minimum of external imports, physical support, and capital outlay, the exact conditions for beekeeping with *A. cerana*. It is easy for an isolated farming community to practice beekeeping with *A. cerana*. Unless the first swarms are purchased, there is no capital outlay, all the materials necessary can be collected locally. Only minimal labor is required for maintenance. The bee does not need to be fed, fumigated, or migrated to warmer areas. Very little development investment is required, essentially only information and training, and as there is no outlay there is no risk involved in failure. Beekeeping can be used as a basis for income generation. *A. cerana* honey has a comparative advantage in terms of quality, and the selling points of being generally “organic” and “natural.” The wax output can be used to support the development of small-scale

organic cosmetics industries in the villages using local herbs and oils as supplements. Where development interventions are focused on the introduction of early-fruiting cash crops like apples, *A. cerana* beekeeping is almost a necessity. It is the only way of ensuring pollination of fruit and vegetable crops grown at higher altitudes and a major adjunct for improving productivity.

A. cerana is affected by

- Changes in mountain farming systems.
- Change in land use patterns and intensification of agriculture.
- Move towards monocultures.
- Loss of wild habitat.
- Increased pesticide use following the introduction of cash crop-based farming systems.
- Changes in habitat and plant biodiversity.

A. mellifera

- Aggressive introduction and promotion of exotic *A. mellifera* by government and nongovernment agencies.
- Diseases and parasites brought in with the exotic bee.

1.11 Strategies to Conserve *A. cerana*

Beekeeping experience and the associated knowledge accumulated by mountain societies through the centuries are valuable assets. Loss of this indigenous knowledge could have drastic implications for beekeeping development and the development of mountain farming. Development organizations and government agencies need to consider carefully under what conditions it is appropriate to promote *A. mellifera*, and when it should be avoided. Many policy-level issues will need to be addressed on a country-specific basis. Beekeeping with *A. cerana* needs to be promoted in the higher-altitude areas where it has the most advantage, and knowledge needs to be reintroduced where it has already been lost. Strains of bees with increased honey production capabilities should be selected out and multiplied, and trials made of beekeeping in lower-lying areas. Beekeepers need to network to exchange experience, microenterprise activities to be explored, and marketing developed. All organizations and institutions concerned with beekeeping and/or the development of mountain farming should include a program on *A. cerana* conservation and dissemination as a part of their packages of materials for higher areas.

- Reorientation of beekeeping trainers, development workers, researchers, and policy makers.
- Facilitation and capacity building of networks and associations of grass roots beekeepers.
- Establishment of country-specific centers for *A. cerana* selection, management, and breeding programs to identify, multiply, and disseminate more productive races of *A. cerana*.

- Development of a mountain-specific extension program on beekeeping development based on *A. cerana*.
- Zonation arrangements for *A. cerana* in inaccessible mountain areas; and banning the introduction and multiplication of *A. mellifera* in such areas.
- Revisiting the definition of agricultural inputs, and incorporating managed pollination through honeybees as an important input in agricultural husbandry.
- Understanding and working to mitigate challenges in the marketing of bee products.
- Policy-level changes towards removing barriers to and facilitating cross-border and cross-continent trade of bee products.
- Promotion of *A. cerana* mountain honey as an organic product.

1.12 Potential of the Indian Hive Bee

Yields from the Indian hive bee are comparable to those from the European bee. In Kashmir, average production of 50 kg of honey per colony is reported (Shah 1981a). There are occasional reports of yields of 50–60 kg of honey per colony in other regions like Bihar, West Bengal, Karnataka, Kerala, and Tamil Nadu, particularly when migratory beekeeping is practiced. This indicates that this bee has the potential for use for commercial beekeeping. Improvement in the hive design, adoption of suitable strain of bee for different agroclimatic conditions, and improvement in management technologies can help to realize this potential. Verma (1989) lists the following advantages of beekeeping with *A. cerana*, compared to that with the exotic bee:

1. *A. cerana* is gentle to handle, industrious, and well adapted to the ecological conditions of south and southeast Asia.
2. It is less susceptible than *A. mellifera* to nosema disease, not seriously affected by *Varroa*, and is less prone to the attack of predatory wasps.
3. To control diseases, parasites and predators, beekeeping with *A. mellifera* requires chemical treatment of colonies. Chemicals are not required in beekeeping with *A. cerana*.
4. The variety of geographical races/populations of *A. cerana* that exists in south and southeast Asia provides excellent opportunities for the genetic improvement of this native species through selective breeding.
5. Through genetic engineering techniques, it may be possible to introduce desirable genes from *A. cerana* into *A. mellifera*.
6. *A. cerana* is sympatric in distribution and can coexist with the two other species of Asiatic honeybees, *A. dorsata*, and *A. florea*, without any adverse ecological consequences.
7. For pollination purposes, *A. cerana* is superior to *A. mellifera* in certain aspects, e.g., it is more suitable for cross-pollinating entomophilous crops grown in the small holdings of this region because of its shorter flight range and longer foraging hours than the European honeybee. Use of bee hives for pollination of agricultural and horticultural crops is another field that is gaining importance in

recent years. There is an increasing demand for bee colonies by orchardists growing apples, litchi, lemon, other citrus fruits, producers of seed of cucumber and other cucurbits, cole crops, spices, onion and vegetable crops and flower seeds, as also by farmers growing sunflower. Payment by the farmer to the beekeeper for pollination service is also becoming a common practice. Bee colonies migrated to farm and orchard areas, to tide over adverse periods, can be utilized for crop pollination. Such migrations can be doubly beneficial to the beekeeper. The overall income from apiculture can thus be quite substantial. Besides honey, the hive bees can produce beeswax, pollen, royal jelly, and bee venom. Technologies exist for beeswax, pollen, and bee venom production. For royal jelly too, suitable technology can easily be developed. Beekeeping for package bee production, queen rearing and supply, breeding and supply of improved strains of bees, and similar nontraditional products can be introduced. Efficient management methods for production of these items can augment the productivity of the bees and losses will be low.

An important feature in which *A. mellifera* differs with *A. cerana* is its use of propolis. Propolis is used to seal cracks and crevices in the nest and make the hive weather-proof. The frames or the inner cover is often joined to the hive body with propolis. This makes inspection or management of colonies difficult. Some races are heavy propolizers. Special techniques have to be adopted to handle the bees without unduly disturbing the nest. Propolis has antibacterial properties. It has several medicinal applications. With the introduction of beekeeping with *A. mellifera* in India, it is now possible to develop suitable technology for production of propolis.

1.13 Commercial Beekeeping

Generally speaking, there are two possible approaches to the development of commercial beekeeping in Asia: the introduction of modern beekeeping with *A. mellifera* or the improvement of existing techniques for using *A. cerana*. Notwithstanding the difficulties involved in establishing new apiaries of the introduced colonies and in developing colony management techniques suitable to local conditions, *A. mellifera* colonies are generally more productive than those of *A. cerana* where forage is abundant, and the development of beekeeping with *A. mellifera* in Japan, the Republic of Korea, China, and northern Thailand is based on this finding.

On the other hand, where forage is available only marginally, colonies of *A. cerana* survive better and can produce with lower management inputs than colonies of *A. mellifera*. It is the absconding behavior of most, if not all, tropical races of *A. cerana* that creates a major obstacle to the development of beekeeping with this bee in rural areas in southern Asia. Since this behavior is apparently triggered, at least to some extent, by an unfavorable hive environment, proper colony management may be able to provide at least a partial solution to this problem.

Despite its economic usefulness, *A. cerana* is not as much studied as *A. mellifera* and there is paucity of comprehensive information on different aspects of this

important honeybee species. In China, out of more than 8.5 million colonies of bees kept in modern hive, 70 % are exotic *A. mellifera*. Similarly, in South Korea, only 16 % beekeeping is with native *A. cerana* and remaining has been replaced by exotic *A. mellifera*. In India, only 10 % of beekeeping is done with *A. cerana*.

To promote beekeeping as a sustainable option for rural development, crop production, and biodiversity conservation, there is an urgent need to generate information on this important species. Although a number of publications have appeared on honeybees in the market, no attempt has been made to approach the subject in a systematic and comprehensive manner in case of *A. cerana*. The aim of this book is to fill the gap by providing detailed information on different aspects of *A. cerana* leading to sustainability and environmental protection. Beekeeping with *A. cerana* supports agricultural production, forestry, and maintenance of biodiversity and natural resources through pollination services. The compilation of this book is unique in the sense that in the context of pollinator decline over the world, conservation of this species will be a step for sustaining food security and issues related to livelihoods.

This book contains 23 chapters and provides complete information on all aspects of *A. cerana* beekeeping. This book is first of its kind, which deals with details on varied aspects of *A. cerana* biology, management, conservation strategies for protecting biodiversity, and enhancing crop productivity. It deals with genetics and breeding of bees, their dance language, foraging behavior, floral sources, communication mechanism through pheromones, molecular phylogeny, impact of invasive bees, safety of bees, declining biodiversity, defensive strategies against diseases and enemies and impact of climate change, food security and livelihood, etc. The book has a wider approach not strictly focused on management of Asiatic honeybee compared to other books on the subject that are strictly oriented towards the management of honeybees but has a generalist approach to different aspects of *A. cerana*.

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Chapter 2

Historical Perspective

2.1 The Origins of Honeybees

Honeybees have evolved from short-tongued, spheciform wasps and first appeared during the Cretaceous period about 130 million years ago. At that time, present-day continents such as Africa, India, South America, Australia and Antarctica constituted a single landmass called Gondwana. Germinating in the warm dry Gondwanan climate, flowering plants called angiosperms developed colours and petal patterns to attract insects, which were more reliable than wind to transfer pollen. In addition to pollen, flowers eventually produced nectar, providing carbohydrates to their winged vectors. About 120 million years ago, the honeybee developed its morphologies specifically to collect pollen and nectar, such as increased fuzziness, pollen baskets, longer tongues and colonies to store supplies.

As Gondwana gradually broke apart and temperatures cooled dramatically during the Oligocene–Miocene about 35–40 million years ago, European honeybees went extinct, while Indo-European honeybees survived and began to speciate. Open-nesting honeybees perhaps evolved before cavity-nesting bees, probably in India, but evidence is still lacking. In any event, a cavity-nesting honeybee spread east and north about 6 million years ago. During a Pleistocene warming about 2–3 million years ago, this bee spread west into Europe and then into Africa to become *A. mellifera*.

It is thought that *Apis florea* and *Apis dorsata* may have existed as separate species as early as the Oligocene period. It has not been possible to estimate when bees of the *mellifera/cerana* type first appeared on Earth. *Mellifera* and *cerana* must have acquired separate identities during the latter part of the Tertiary era. The two species were apparently physically separated at the time of the last glaciation, and there was no subsequent contact between them until that brought about by human intervention in recent times. In the post-glacial period, *mellifera* and *cerana* have shown similar evolution into geographical subspecies or races.

2.2 The Development of Subspecies

Although it has long been known that there are many kinds of honeybees, and these have been the subject of scientific study for more than two centuries, only in recent years has a comprehensive classification been attempted, which takes into account not only differences in physical characters between subspecies and their present geographical distribution but also the geological evidence pointing to their origins and to the course of their subsequent evolution and distribution.

Like the stingless bees, honeybees first evolved in tropical conditions. The fossil record shows that at the time the area of land that is now Europe had a tropical climate. As the climate became cooler, the open-nesting types would not have been able to survive except by migrating to the tropical region of Southern Asia. For the greater part of the Tertiary era, Africa was isolated from Europe by sea, and no Tertiary types of honeybee reached Africa even after a land bridge was established. It is likely that the development of advanced thermal homeostasis in honeybees, which permitted the occupation of cool temperate zones, therefore occurred in Southern Asia, possibly in the Himalayan region. Once established, the cavity-nesting *cerana*-*mellifera* type would spread East and West, eventually occupying both tropic and cool temperate zones.

A physical separation into two groups probably took place as a result of the glaciations, which occurred during the Pleistocene period (1 million to 25,000 years ago) and desert and semi-desert then kept the two groups separate during intervening warm periods. Thus, *mellifera* and *cerana*, although originating from a common stock, evolved into distinct species. The ultimate Western boundary of the *cerana* territory was in Afghanistan some 600 km to the East of the nearest *mellifera* colonies in Iran. The *cerana* territory comprised the Indian Subcontinent south of the great mountain ranges, Ceylon, Malaysia and Indo-China, and the East Indies including the Celebes, Timor and the Philippines. In Eastern Asia, it reached a latitude of 46 degrees, and occupied Japan except for the island of Hokkaido. *Mellifera* spread westwards through Asia Minor to colonise the Balkans and the Mediterranean region and southwards through the Arabian Peninsula to occupy central and southern Africa. Similarities between neighbouring subspecies suggest that the Iberian Peninsula and southern France were colonised from North Africa.

How far *mellifera* bees may have penetrated into northern and western Europe during the warm intervals between the glaciations of the Pleistocene period can only be a matter of conjecture; what is certain is that no honeybees could have existed north of the Mediterranean region, the Iberian Peninsula and southwestern France at the time of the most recent Ice Age. Although at its maximum extent in western Europe some 18,000 years ago, the ice sheet only reached as far as northern Britain, the area for hundreds of miles to the south was inhospitable tundra.

In the warm period, which followed the Ice Age (starting about 14,000 years ago), the ice sheet gradually retreated and the tundra was replaced by forests of birch, pine, hazel, elm and broad-leaved oak. The western honeybee was once more able to extend its domain in Europe. In the east, advance beyond the Caucasian region proved

impossible, owing to the lack of suitable nesting sites in the steppes of southern Russia. The bees of the Balkan area spread northwards to occupy the eastern Alpine valleys, central Europe as far as the 50th parallel of latitude, and the western shores of the Black Sea. In the West, the bees, which had found refuge in southern France during the Ice Age, spread across Europe north of the Alps, eventually occupying an area from the Atlantic seaboard to the Ural Mountains. The northernmost limit of the territory may have been in southern Norway; honeybee remains dating from c. 1200 have been found in an archaeological dig in Oslo, although honeybees had not been reported in Norway prior to the nineteenth century. The mountain ranges of the Alps and the Pyrenees obstructed the northward movement of the bees in the Italian and Iberian peninsulas.

In colonising this vast territory, stretching from the Urals to the Cape of Good Hope, *A. mellifera* had to adapt itself to a large variety of habitats and climates ranging from the continental climate of eastern Europe with its harsh winters, late springs and hot, dry summers, through Alpine, cool temperate, maritime, Mediterranean, semi-desert and tropical environments. This adaptation was achieved by natural selection, producing some two dozen subspecies or races. All the subspecies of the *mellifera* group can interbreed given the right conditions, but the crosses show hybrid characters.

Although *cerana* bees must have shared a common ancestor with *mellifera*, they have evolved into separate species. It is not possible to cross *cerana* with *mellifera* even using instrumental insemination, because the two species are now genetically incompatible and viable eggs do not result from the cross-fertilisation. Other differences include their differing reactions to diseases, infestations and predators. *Cerana* can tolerate varroa and has developed an effective defence strategy against the Giant Hornet, against which *mellifera* bees have no defence. *Cerana* is, however, highly susceptible to the acarine mite, which arrived with the introduction of *mellifera* bees into *cerana* territory. It is also highly susceptible to sac brood and foulbrood, but not markedly so to nosema.

The different races of *A. mellifera* can generally be differentiated in physiological terms. Bees from warmer climates tend to be smaller in size and lighter in colour than those adapted to the colder regions, although this rule is not invariable. The effect of altitude seems to be similar to that of increasing latitude. Accurate differentiation between races of similar appearance requires precise morphometric examination of representative samples of bees. There are also differences between races in natural history and biology. Some subspecies are more prone to swarming than others, some produce large numbers of young queens when swarming, others only a few. Tropical honeybees frequently 'abscond' or migrate, sometimes because of lack of forage through drought or other causes, perhaps as a defence against predators. Heavy predation is also a likely cause of the vigorous defence reaction of some races, for example, the bees of tropical Africa.

The bees of the warmer regions do not need to cluster as tightly as those confined to the nest through long, cold winters. Brood rearing is adapted to take maximum advantage of the local flora. Where bees of the same race have occupied different kinds of habitat, they have formed local strains which have accommodated themselves to

the different conditions. Similarly, honeybees of different races which have occupied similar habitats have evolved similar behavioural characters. Even the 'dance language' by which honeybees communicate information about the location of food sources may differ in detail between races, as different races may be conditioned to foraging over different distances from the nest. (Professor Goats described these differing dance patterns as 'honeybee dialects'.) The behavioural characters of the different races and strains, brood rearing pattern, foraging behaviour, clustering, etc., are fixed genetically, so that a colony cannot readily adapt itself when transferred to a different kind of environment.

The dark European honeybee, *A. mellifera mellifera*, is fairly uniform over its whole range, having had but a comparatively short time in which regional varieties could evolve, but even in this race, differences can be observed between strains. In France, where the bee has been domiciled longest, there are distinct differences in brood rearing pattern between the *mellifera* bees of the Landes district in the southwest, the bees of the Paris area and those of Corsica. The Landes bees are typical 'heather bees', conditioned to a principal nectar flow in late summer and early autumn. In the Paris area, there is no summer nectar flow and the bees show early spring brood activity. Exchange of colonies between the Landes and Paris resulted in poor performance in both cases. In Corsica, the *mellifera* bees follow a Mediterranean pattern with little or no brood production in summer and a second peak in autumn.

The behavioural patterns which have evolved in the different races have ensured the survival of the various subspecies in their native habitats, and some of these patterns may be repeated in different races. There is one race which, in spite of small economic importance, possesses an apparently unique biological character, which renders it of great importance in the study of the genetics of honeybees. In all other races, when a colony is rendered queenless, laying workers may appear which are capable of laying drone eggs only. In *A. mellifera capensis*, the Cape bee, when a colony is deprived of its queen, a laying worker appears within a few days which, for a period, is able to lay predominantly diploid worker eggs. From these eggs, true queens capable of being mated can be raised, re-establishing queenrightness in the colony.

The genus *Apis* can be divided into three branches based on their nesting pattern: the open-nesting single-combed giant honeybees *A. dorsata* and *Apis laboriosa*; the dwarf honeybees *A. florea* and *Apis andreniformis*; and the cavity-nesting *A. cerana*, *Apis koschevnikovi*, *Apis nuluensis*, *Apis nigrocincta* and *A. mellifera*. All the nine species thrive well in environmental extremes like deserts, rain forests and tundra, but most people are familiar with *A. mellifera* only, which is known as important pollinator for agricultural production all over the world.

Early civilizations quickly mastered honey hunting skills, shown in rock art in Africa, India and Spain. Egypt, Greece, Italy and Israel developed organized beekeeping centres until the Roman Empire dissolved in approximately 400 AD. Christianity monasteries and convents then served as apiculture centres until Henry VIII closed them at the beginning of the Reformation. Science and technology provided the next insights into apiculture during the Enlightenment.

Honeybees expanded to North America with human-assisted migration during the seventeenth century. Many Europeans fleeing wars, poverty, land laws or religious persecution brought extensive beekeeping skills to the USA during the next two centuries. Meanwhile, English colonists took bees to New Zealand, Australia and Tasmania, completing human-assisted migration of *A. mellifera* around the globe.

Beekeeping became commercially viable during the nineteenth century with four inventions: the movable-frame hive, the smoker, the comb foundation maker and the honey extractor. These inventions still support commercial apiculture. A fifth invention, a queen grafting tool, allows beekeepers to control genetic lines.

Honeybees are such efficient pollinators that industrialized countries developed specialized agriculture dependent upon migratory pollination with honeybee, *A. mellifera*. The US Congress passed a Honey Bee Restriction Act in 1922, to protect *A. mellifera* from the damage that tracheal mites were doing to honeybees in Europe, until tracheal and varroa mites arrived in the 1980s, resulting in 50–80 % loss of their colonies.

Different honeybee races can clash with pre-existing insect species. In the 1950s, the honeybee *A. mellifera scutellata* (one type of African honeybee) was taken to Brazil via human assistance, creating ramifications for the endemic bee species in both South and North America. Similarly, *A. mellifera* was introduced to India and China, but it competes with the smaller *A. florea* for floral sources.

Honeybees can adapt to minor changes in global warming, but colony collapse disorder is the most recent bittersweet reminder that human society threatens honeybee habitats and breeding patterns on a global scale. Promoting genetic diversity of honeybees and providing safe environments are crucial steps towards future sustainable agriculture.

2.3 Knowledge About *A. mellifera*

A certain amount of knowledge about *A. mellifera* in ancient Egypt is attested by depictions of the bee and of hive beekeeping found during excavations. Statements about honey and beeswax survive from Egypt, Greece and Rome, but most of them record trade in these goods, or their use as offerings, gifts or the payment of dues.

2.3.1 Ancient Egypt

From about 3100 BC, the profile of the worker honeybee (*A. mellifera*) was used as a hieroglyph in the topographical symbol of ancient Egypt. The earliest examples excavated show four of the six legs and two of the four wings. The bee's head, thorax and banded abdomen were demarcated, as well as the two antennae and four legs. Hives of bees were portrayed by around 2400 BC. Four early representations of honey harvesting from hives have been found during the excavations in Egypt

Fig. 2.1 An early representation of a honeybee: a stone bas-relief from the Sun Temple of Ne-user-re, Abu Ghorab, Lower Egypt, c. 2400 BC (drawing: Egyptian National Museum Catalogue). (Crane 2004)



(Crane and Graham 1985). One of them, reproduced in Fig. 2.1, shows that smoke was used to pacify bees. Also, as combs taken from the hive were round in shape, the beekeepers who produced them must have known how to get the bees to build their combs directly across the horizontal cylindrical hives shown. The beekeepers also probably understood—as traditional beekeepers do today—that the bees build their combs in a nest cavity or hive at a constant separation. Thus, if two or more guide combs are inserted in an empty hive, they must be separated at the bees' comb spacing. Traditional beekeepers in Greece use the combined width of the first two fingers, or the length of the distal (outer) thumb segment, to determine the distance between the midribs of adjacent combs. Although no written descriptions of bees or beekeeping are known from ancient Egypt, the depictions suggest that beekeeping methods reached a higher level there than anywhere else at the same period (2400–1400 BC). The method used by traditional beekeepers in Upper Egypt today is similar to that depicted in 1450 BC (Fig. 2.2). The method was also transmitted westward along the north African coast and to Sicily, and some, but not all, parts of it reached Greece and Rome.

Fig. 2.2 Part of the wall-painting in Rekhmire's tomb (no. 100), West Bank, Luxor, Upper Egypt, c. 1450 BC (Davies 1944; Crane 2004)



2.3.2 Ancient Greece

In ancient Greece, it was known that each colony contained one larger bee, which was regarded as the ruler and assumed to be male. Early Greek texts have praised the large bee in the hive for its outstanding leadership abilities and wisdom. Some of the texts praised the bee's feminine gender characteristics and others praised its masculine ones—using almost identical terms. In either case, all the other bees were subject to their leader, from whom they did not want to be separated. These authors concluded that praising bees was a common rhetorical device of classical Greek writers, who were not concerned with the gender of the bees' ruler. Writings on bees, which survive from ancient Greece, contain a number of important statements. They describe behavioural characteristics of *A. mellifera* more or less correctly, although the physiological basis for these was usually not understood. Books I to VIII of *Historia animalium* were written by Aristotle (384–322 BC), but the large amount of information on bees is mostly in Book IX, whose author is unknown. The following statements are quoted or summarized from Book IX, which state that outside the colony, bees visit flowers, but only one kind on the same trip. They collect propolis, 'the "tears" or exuding sap of trees ...' and use it to 'narrow ... the entrances to the hive if they are too wide'. 'Others (of the foragers) carry water' and bees 'discharge their excrement in flight'. When drones fly out 'they soar up in the air in a stream, whirling round and round'. Regarding swarming: 'They say that, if a young swarm goes astray, it will turn back upon its route, and by the aid of scent seek out its leader'. 'In the colony, there is a division of work in the hive: some make wax, some make honey, some make bee-bread (pollen), some shape and mould combs ...'. 'Others smooth and arrange combs'. Bees 'store up another article of food resembling wax in hardness, which by some is called *sandarace*, or bee bread. This they carry on their legs'. 'When the floral world is in full bloom then they make wax' and when bees are smoked they 'devour the honey most ravenously'. They build cells 'for the Kings only when the brood of young is numerous' and the 'bees that die are removed from the hive'. 'When honey runs short they expel the drones'.

In the 100s BC, Zenodorus of Sicily proved that, of the three regular figures, which will completely fill an area, the hexagon (the cross-section of a bees' cell) has the greatest surface area (Betts 1921). From the Hellenistic Period (323–331 BC), the concept of *bugonia* (birth from an ox) was prevalent; it was probably of Egyptian origin. To produce a swarm of bees, an ox was to be beaten to death without breaking the skin and the body to be enclosed with herbs in a special building for 9 days, after which a swarm of bees would appear. The idea probably arose from a confusion between the honeybee and the drone fly *Eristalis tenax*, and between larvae of the honeybee and of the blow fly (*Calliphora* spp.). Other misconceptions have been listed elsewhere (Crane 1999), many of them could have been corrected by quite simple observations. Davies and Kathirithamby (1986) included a full discussion of knowledge about insects in ancient Greece, and of the attitude to studies of such small animals.

2.3.3 Ancient Rome

Roman writings include many statements similar to those in Greek texts (Crane 1990) which supports the view that Greece was the source of Rome's knowledge of bees. Roman authors wrote much about bees and beekeeping, and the main passages by the following authors such as: (1) Varro (116–27 BC) *Res rusticae*, Book III.16.1–382; (2) Virgil (70–19 BC) *Georgics*, Book IV; (3) Columella (c. 60 AD) *De re rustica*, Book IX.2–16; (4) Pliny (the Elder) (23–79 AD) *Naturalis historia*, Book XI, 4–16; XXI, some of 43–49; (5) Aelian (died c. 220 AD) *De natura animalium*, scattered references; and (6) Palladius (300s AD) *Opus agriculturae*, scattered references were quoted by Crane (1994) with short commentaries.

2.4 Knowledge About *A. mellifera* up to 1500–1600

A number of the writings about bees from other Mediterranean civilizations have been lost, but several were translated and preserved by Arab scholars who lived in Spain during the period between the invasion by Muslims in 711 and their expulsion in 1492. Some writers who lived between the 900s and 1100s were especially important in preserving knowledge about bees, and in adding new knowledge (Monferrer 1991). They include the following: Ibn Sina (980–1037) or Avicenna, who was born near Bokhara in Uzbekistan and died in Persia; Ibn Wafid (born in 1008) of Toledo, who studied in Córdoba; Abu Zacaria (1000s–1100) in Tunis; Ibn-al-Awam of Seville; and Ibn Ruoshd (1126–1198) or Averroës, born in Córdoba.

Avicenna (unlike Aristotle) knew that 'kings' were reared in extra large cells. Ibn-al-Awam stated that the smallest bees in the hive are females, which have a sting. Larger bees are males, which take no part in the preparation of honey. The 'kings' are twice as large as the bees that make honey, and Ibn-al-Awam knew that

it was advantageous to the beekeeper to have only a small number of these in a hive (Bee World 1932). Many Greek writings were translated into Arabic; from these, Latin translations were made and later disseminated widely in Europe. Books were printed in Europe from 1459 onwards. In 1513, Gabriel Alonso de Herrera in Spain published a compilation of writings on agriculture by previous authors (Alonso De Herrera 1513) and Volume 5 of this work was devoted to bees. It reported what Greek and Roman authors had written.

In 1586, a book by Luiz Méndez de Torres in Spain contained the following explicit statement: . . . la aveja, que dicen maessa, o maestra, sin ayuntamiento de macho, y sin dolor, echa de si una semilla, de que se engendran tres generos de avejas, que son, maestras, y zanganos, y avejas. De suerte, que siendo la simiente una misma, por razon de la diversidad de los vasos donde se pone . . . (. . . the bee, called the maessa or maestro (mistress), without coupling with a male (this is incorrect) and without the pain of childbirth, produces a seed from which are engendered three kinds of bee—maestras, drones and ordinary bees—according to the different cells in which the seed is placed. . .) (Méndez 1586).

Meanwhile, in 1568 in Silesia, Nickel Jacob had published a book on beekeeping (Jacob 1568) which included two significant new observations: (a) a colony with (or given) young worker brood or eggs can rear a new *Weisel* (a masculine noun used for the ruler of the colony and (b) when a hive of bees is put in a new place, the bees learn its location by circling in the air above it. Important advances followed after Galileo (1564–1642), in Italy, developed a compound microscope; he described it in 1610, although the word *microscope* was not in use until 1624. But before that, Giovanni Rucellai (1475–1525, also in Italy) wrote a poem *Leapi*—not published until 1539—which described what he had seen of the bee’s external morphology, including the proboscis and sting, by using a concave mirror (*specchio lucido escavate*). Rucellai referred to the ruler as king (*re*), never as queen (*regina*). Galileo was a member of a small but active scientific society in Rome, the Accademia dei Lincei (Academy of the Lynxes) (Carutti 1883). In 1624, he gave a microscope to Prince Federico Cesi, the founder of the Academy, who used it to draw honeybees on a broadsheet to be presented to the Pope (Fig. 2.3); they were the first insects to be depicted as seen under a microscope (Crane 1963). Cesi also started to edit his large drawings to produce a textbook on bees, *Apiarium*, which was to be indexed and made suitable for quick reference (Alessandrini 1956). He cut up the text, stuck each item on a separate sheet of a notebook and wrote notes and additions in the margins. His draft can still be seen in the Academy in Rome, but Cesi died before he could complete the book.

2.5 New Knowledge About *A. mellifera* between 1630 and 1800

2.5.1 Observation Hives

In 1654 in England, John Evelyn was shown ‘Transparent Apiaries’ in the garden of Dr. Wilkins at Wadham College, Oxford, although the ‘glass hive’ he drew appears to have only small windows (Smith 1965). The diary of Samuel Pepys for 5 May

Fig. 2.3 Broadsheet (100 cm × 64 cm) presented to Pope Urban VIII in 1625, with the first drawings of bees made using a microscope. (Crane 2004)

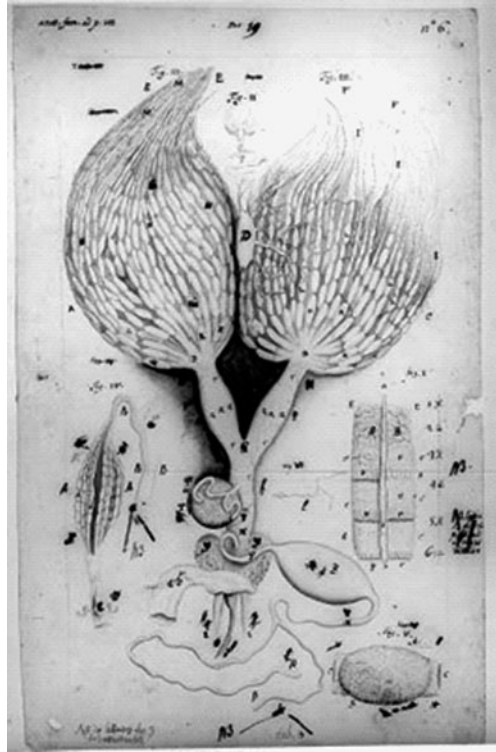


1665 refers to bees ‘being hived in glass (so that) you may see the Bees making their honey and combs mightily pleasantly’. By about 1670, large sheets of glass were available in England, and observation hives could then be made with glass sides. The use of such hives for studying the activities of bees seems to have developed rather slowly, although Réaumur (1740) believed that Swammerdam must have used one in his studies of bees between 1669 and 1673. Robert Boyle, an Irish chemist and physicist, said in 1688 that he had in his closet ‘a Transparent Hive, whence there was a free passage (for the bees) into a Neighbouring Garden’ (Boyle 1688). In 1712, the Italian astronomer Maraldi referred to his observations using a large number of glass hives in a garden in Paris (Maraldi 1712), and in the same year, Joseph Warder in England reported keeping bees in ‘transparent boxes’ (Warder 1712). In 1788, Spitzner described his observation hive, which held a single comb between two large sheets of glass, and he stated that returning foragers danced on the comb surface (Spitzner 1788).

2.6 Reproduction and Gender in Honeybees

The statement by Luis Méndez de Torres in Spain in 1586, that the ruler bee was female and the mother of all the other bees in her colony, slowly filtered through to other countries. In 1637 in England, Richard Remnant wrote that ‘the females (from

Fig. 2.4 Reproductive apparatus of the queen honeybee, drawn by Swammerdam in 1669/1673. Part of the sting apparatus is below it. (Crane 2004)



the context, queens) have near their stings a little neat place of receipt for generation' (Remnant 1637). Between 1669 and 1673 in the Netherlands, Swammerdam drew the queen's reproductive system as he saw it under the microscope (Fig. 2.4). But he died in 1680, and his study on honeybee anatomy was first published in 1737/1738, in Dutch and Latin; an English translation followed in 1758 (Swammerdam 1737/1738). Meanwhile, John Thorley in England (1744) had seen that 'a little Worm or Maggot' which had developed from the object a ruler bee deposited on his hand; this was therefore an egg, not sperm or semen (Thorley 1744).

In 1609, Charles Butler, in England, had argued that the drone was 'the male bee'. Then, in 1637, Remnant said that 'there is in the hinder part of the male or Drone a little white thing like the instrument of generation; take one of them alive and crush the body of it somewhat hard between your fingers, and you shall see it put forth'. In 1712, Joseph Warder published proof of this. 'Let any Gentleman (whose Curiosity leads him to know the Truth), but gently cut up with a Launcet . . . the hinder part of the Drone-Bee, there he shall find . . . a large pair of testicles, as big as great Pins Heads, Milk white . . . the *Penis* . . . in shape much resembling the Head of a Bullock with its Horns' (Warder 1712). (The drone has an internal endophallus that is everted in mating.) Volume 5 of Réaumur's great book on insects (1740) illustrated the reproductive systems of the queen and drone honeybee. He referred

to ‘observations made more than 100 years ago (presumably by Méndez de Torres in 1586) which showed that this bee . . . which the Ancients called the King bee . . . is a female’. In 1771, Anton Janscha, in Austria, established that a young queen honeybee mated in flight, usually while out of sight (Janscha 1771). On her return, as a sign of mating, ‘something white, like a thin thread, hangs from it (the hinder part of the abdomen), and appears as though injured or torn. She will begin to lay eggs in five or six days’. (The returning mated queen carries in her vagina part of a drone’s everted genitalia that become detached during mating.) In 1792, François Huber, in Switzerland, showed that workers were females with undeveloped ovaries, which were unable to mate and could lay only unfertilized (drone) eggs (Huber 1792). By using a glass hive containing three combs, Huber established that the (mated) queen of the colony left with a swarm, and that a newly reared (unmated) queen left with any afterswarm. As a result of his own observations, Dzierzon in Silesia proposed in 1845 that queens and workers develop from eggs fertilized by sperm from a drone the queen (mother) had mated with, whereas drone offspring develop by parthenogenesis from unfertilized eggs (Dzierzon 1845).

2.7 Communication by ‘Dancing’

The major research on honeybee dances by Karl von Frisch (Fig. 2.5) was carried out in the early 1920s. It was described in full in his 1965 book, and later summarized in English in 1969. The dances had been observed much earlier, although not studied. For instance, in around 1655, John Evelyn, in England, had watched dances of scout bees sent out from the swarm cluster, ‘*tanquam explorates*’. In 1952, Rosamund Duruz found a description of them in Evelyn’s *Elysium Britannicum*, and David Smith published this in *Bee World* in 1965. Evelyn wrote that ‘by a kind of shivering motion’, these bees communicate information about a new nest site ‘to the whole swarm and centre bees in a moment, at which signal they dissolve the populous and moving cone, and fly immediately to the place’ (Evelyn 1655).

2.8 Beeswax, Nectar, Honey and Pollen

Studies of bee products became more common after 1700. Beeswax had sometimes been confused with the pollen that bees collect from flowers and carry to the hive on their hind legs. Thus, it was not realized that the bees actually secrete the wax. However, in 1684, Martin John, in Germany, reported that he had observed wax scales on the abdomen of workers (John 1684). Then, in 1744, Hornbostel (also in Germany) stated that beeswax came ‘from the body of the bee’ (Hornbostel 1744), and John Thorley, in England, noted wax scales in ‘pockets’ under the worker’s abdomen (Thorley 1744). In 1792, the English surgeon John Hunter described his observations on a colony in a glass hive (Hunter 1792) including the production of

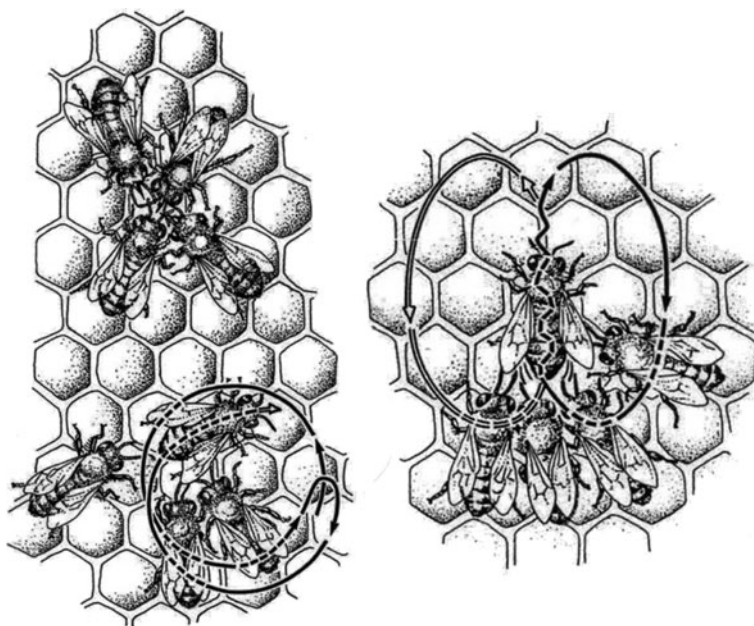


Fig. 2.5 Round and wagtail dances of the honeybee studied from 1920 onwards (von Frisch 1969; redrawn by R. Lewington. The bee performing a round dance (*left*) is followed by two bees, and the bee doing a wagtail dance (*right*) by four. (Crane 2004)

wax by worker bees. He concluded that ‘the wax is formed by the bees themselves; it may be called an external secretion of oil’. In the same year, François Huber, in Switzerland, observed and described how the bees constructed their beeswax combs (Huber 1792).

Until 1700, it was believed that the sweet liquid that the bees collected from flowers fell on to the flowers from heaven. For instance, Charles Butler (1609) said: ‘The greatest plenty of purest nectar cometh from above; which Almighty God doth miraculously distil out of the air . . . the very quintessence of all the sweetness of the earth, drawn up, . . . and condensated by the nightly cold into this most sweet and sovereign nectar, which thence doth descend into the earth in a dew or small drizzling rain’. During the 1700s, it was shown that nectar does not fall from the skies, but is produced in the nectaries of flowers. A lecture by Vaillant in 1717 on the structure of plants (Vaillant 1718) was published in 1718, and has been cited by several writers (including Bonnier in 1879) as stating the true origin of nectar and also giving the name *mielliers* to nectarines which produced it. However, Vaillant did not mention the organ producing nectar. In England, John Hill wrote in 1759 that nectar is ‘produced within the flowers, where it is found’, but I do not know whether this was an independent observation (Hill 1759). Only after 1800 was it discovered that the sugars in honey are different from those in nectar. A salivary

secretion produced by the bees contains enzymes that convert sucrose in nectar into glucose and fructose.

2.9 Pollen and Pollination

The word pollen was first used in English in 1686 by John Ray (1686) who has been called the father of English natural history. However, the older names ‘bee bread’ and ‘*farina*’ (Latin for flour) persisted. Arthur Dobbs, in Ireland, used the latter in 1750, in the earliest description found of the part played by honeybees in plant pollination (Dobbs 1750): ‘From the late Improvements made by Glasses, and Experiments made, . . . it is almost demonstrable, that the *Farina* upon the *Apices* of flowers is the Male Seed; which entering the *Pistillum* or *Matrix* in the Flower, impregnates the *Ovum*, and makes it prolific. . . . Now if the Bee is appointed by Providence to go only, at each loading, to Flowers of the same Species, as the abundant *Farina* often covers the whole Bee, as well as what it loads upon its Legs, it carries the *Farina* from Flower to Flower, and by its walking upon the *Pistillum* and Agitation of its Wings, it contributes greatly to the *Farina*’s entering into the *Pistillum* . . .’.

2.10 Early Knowledge About Other *Apis* Species

A. cerana, the hive bee in Asia east of Iran, is slightly smaller than *A. mellifera*. In the wild, it often nests in a tree cavity. The earliest references found to the use of hives for *A. cerana* date from the 300s BC in the Upper Indus basin, and from the 200s AD in China and Vietnam (Crane 1999). Beekeeping methods changed little over the centuries, until adapted movable-frame hives were introduced from Europe in the late 1800s.

In China, Chang Hwa’s *Natural Science Book* dating from 265 to 290 AD described a practice used in places ‘beyond the distant mountains’, when swarms of *A. cerana* were seeking nest sites: ‘People use wooden vessels and bore holes in them, and rub beeswax on the inside and outside of the vessel. They catch several bees into the vessel in spring time, and a moment later let the bees fly away, then these bees will accompany a lot of other bees to the vessel, and after two or three days the vessel contains many bees, and the people take it home’. (Translation by Kenneth Tsang 1979.)

This practice must have been based on a knowledge of the behaviour of swarming bees. Crane (1999) reported that hill people in the forested mountains of Vietnam near its present border with China followed a similar custom. The annals of Nam-Tâm in Vietnam dating from the 700s (Crane 1999) include an account of beekeeping which shows that the bees in an *A. cerana* colony were understood to some extent. The bees were said to have a *chua* or king (queen), to whom the *quân* or soldiers (workers) and the *con tuong* or chiefs (drones) showed entire fidelity. Although

Nam-Tâm stated that ‘the great scholars of Antiquity affirmed that this was true’, but original records are not available. The large ruler bee produced young rulers in special cells. Destroying these cells could control swarming. If the ruler was lost, the bees dispersed. If a swarm had a ruler with it, the bees did not sting. Drones were produced in March/April and disappeared in September/October.

In addition, Nam-Tâm said that pollen from orchids was collected as special food for the queen. A 1773 encyclopaedia by Le Quy Don in Vietnam (*Van dai loai ngu*) included the passage: ‘Bees carry normal pollen to the hive on their legs, but delicate pollens, reserved for the queen, cover their heads’. This statement, together with a mention of orchid pollen during the 700s, suggests that pollinia were observed; these are sticky masses of pollen produced by some orchids, which become attached to the head of a pollinating insect.

Also, in the 700s, a nest of *A. cerana* was apparently used in China for diagnosing human diabetic glycosuria. A sample of the patient’s urine was placed near the nest and a watch kept to check whether the bees were attracted to it; if so, its sugar content was abnormally high (Needham 1970).

In tropical regions of Asia east of Iran, honey and wax were harvested from *A. dorsata* and *A. florea*, two species of honeybee which build a single-comb nest in the open and cannot be kept in a hive. Chang Hwa wrote as follows about these bees (Crane 1999). ‘In far off mountains . . . of the South (their combs) stick to dangerous and perpendicular cliffs which are impossible to climb. . . . After the bees have left the place, some of the wax still sticks to the face of the cliff. A kind of bird, the size of a sparrow, comes in flocks to clean the place by picking up the remaining wax. They are called “spiritual birds”. The next spring the bees come back and make their nests as usual’. The birds were probably the honey guide, *Indicator xanthonotus*. It eats and can digest beeswax, and each male defends a territory that includes one or more *A. dorsata* nests (Friedmann 1955).

2.11 Post 1800

After 1800, the number of studies on honeybees and their products increased more and more rapidly as new scientific equipment and methods became available, and it is not feasible to make a useful summary of them here. The genus *Apis* has an Indo-Malayan origin where both diploid (*A. florea*, *A. dorsata*) and earlier tetraploid (*Apis indica*) species first appeared. During its northward migration by various land routes across the Himalayan barrier, *A. indica* seems to have gradually differentiated into *A. mellifera* and a number African, Eurasian and Sino-Japanese races. Many of these races have accumulated enough genic differentiation and sexual as well as behavioural isolation mechanisms so as to deserve recognition or creation of new species among the tetraploid (Alonso De Herrera 1513). The interpretations of the evolution of *Apis* species have been largely based on morphometric analysis and paleogeography (Bee World 1932).

2.12 *A. cerana*—The Historical Perspective

The earliest literary sources concerned with *A. cerana* beekeeping come from the Rig Veda Canon. The Aryan warriors, at the time of expansion from the Indus area, had the knowledge about honey hunting, but they became skilful beekeepers only gradually when they moved towards the Himalayan valleys. In the forests there, *A. cerana* colonies were most likely in great abundance. In that time, the knowledge about bees was not limited to recognition of two kinds of honey: from *A. dorsata* and *A. cerana*. It was known that combs consist of hexagonal cells placed in rows and that honeycomb with ripe honey is sealed. The Aryans made observations where bees usually settle in natural environment. They considered that the bee colony reflected the hierarchy of the kingdom of man and that the bee sting (which was easy to see when in direct contact) and the stinging organ are located in abdomen. Swarms have been decoyed to skeps where fragments of wild combs were fixed. From every strong *A. cerana* colony, they have taken up to four combs (probably per year). The rest was left for bees. Swarming bees were usually sprinkled with water. Techniques of management and beekeeping equipment were improved gradually.

According to development of medical sources, four kinds of honey were known. In treatises by Sasruta and Charak, called fathers of Ayurveda and Surgery, we can find information that honey from *A. cerana* causes heating when applied directly. It should be used in the case of colds and tuberculosis and as a metabolism-activating factor. *A. dorsata* honey was regarded by them as comparable and was used in treating similar diseases. Gradually, as much as eight kinds of honey were known. The base of differentiation was the colour and rate of the process of granulation. Ancient scientists had noticed that some honeys changed their properties when granulated and after heating. They can even be toxic (Prakash 1961).

In the post-Vedic Period, the main literary achievement of which is Ramayana, we have remarks about specialist apiaries—‘bee gardens’ with a lot of colonies in one place (probably hived in skeps). Beekeeping was one of the popular crafts. If we believe that Ramayana is the earliest literary source of that period, in which beekeeping is not mentioned, we can interpret it as showing gradual decrease in the Aryan’s interest in beekeeping. It should be remembered that honey is no longer the only sweet substance known, but is still indispensable in rituals. In everyday life, it is replaced by sugarcane products (Prakash 1961). Techniques of purifying sugar were also known. The Hindi word *madhu* (honey) remains as a root of various words describing ‘sweetness’. Centuries later, we can only speculate about real ingredients of particular food articles, whether they were made with the addition of honey or sugar. In early Buddhism and Jainism, honey was quite popular, but increasing sugarcane cultivation caused it to be the main source of sweetness (Prakash 1961). In both traditions, descriptions of sugar use predominate, and is most important also, in rituals and religious ceremonies.

2.12.1 *A. cerana* Group

The common name for *A. cerana* is the Asian hive bee. It is sometimes incorrectly named *A. indica*, a classification that is now historic. *A. cerana* is indigenous to Asia between Afghanistan and Japan, and occurs from Russia and China in the north to southern Indonesia. *A. cerana* has been introduced recently to Papua New Guinea and the Solomon Islands. *A. cerana* builds a nest consisting of a series of parallel combs, similar in style to *A. mellifera*, and builds its nest within a cavity. As with *A. mellifera*, *A. cerana* occurs over a huge geographical area, and it varies in size throughout its range: tropical races are smaller bees, with smaller colonies. *A. cerana* bees are smaller than *A. mellifera*, and they form smaller colonies than *A. mellifera*. The foraging range may also be smaller. There are many different races of *A. cerana*, as could be expected from the wide range of habitats it occupies from temperate mountain regions to tropical islands. It is possible that some races of *A. cerana* will in the near future be recognised as separate species. Other honeybee species in Asia showing behaviour similar to *A. cerana* are *A. koschevnikovi*, *A. nigrocincta* and *A. nuluensis*. These gentle species of bees have long been managed as useful honeybees in many parts of Asia and their honey and wax is valued.

A. cerana does not produce propolis. Because it is a cavity-nesting bee, it is possible to keep *A. cerana* in a wide range of hive types and movable-frame hives and movable-comb hives (top-bar hives) have been developed for *A. cerana* and the other cavity-nesting hive bees. Their gentle nature allows these bees to be kept close to home, and in many places, these are kept in logs hanging from the eaves of houses or in specially designed cavities built into the walls of houses.

This chapter represents the first to trace the general history of beekeeping with *A. cerana*, and there are still many gaps in the story which readers will try to fill in. Six possible stages in the development of beekeeping in Asia are briefly summarised in Table 2.1.

Stage I is the collection of honey from wild nests of bees, and I believe that this stage has occurred almost everywhere in the world where there has been honey storing, except where religious ordinance prohibited it. The earliest direct evidence is provided by rock paintings (Table 2.2). These have been found during the present century in all continents except the Americas (Crane 1983); the most recent finds in Asia have been by Mathpal (1984), but these show *A. dorsata* and not *A. cerana*.

Stage 2 is the ownership of wild nests in trees or rocks. In the forests of northern Europe, tree beekeeping occurred from 2000 or 1000 BC to 1700 AD or later (Crane 1983). A beekeeper owned certain trees that contained a wild nest of *A. mellifera*. He looked after the colonies to a certain extent, and in later centuries, fitted a door to get at the honey. The total amount of honey obtained was large—in the 1100s it was over 6,000 t a year in Russia alone (Galton 1971). Vestiges have recently been found of a similar practice among native peoples of northwest Australia, with nests of stingless bees in trees (Dollin and Dollin 1986). *A. cerana* nests have been similarly owned and tended in this way in Laos in southeast Asia. *A. dorsata* nests were certainly owned, for instance, in Sri Lanka. Seligmann and Seligmann (1911)

Table 2.1 Possible stages in beekeeping in different parts of Asia. (Crane 1995)

Stage Dates		Activity
1	Early man to present	Collecting honey from wild nests. Order of importance = <i>A. dorsata</i> (including <i>laboriosa</i>), <i>A. cerana</i> (including <i>A. koschevnikovi</i>), <i>A. florea</i> (including <i>A. andreniformis</i>), Meliponinae (stingless bees)
2	?	Ownership of wild <i>A. cerana</i> nests in trees of rocks (e.g. tree beekeeping)
3	? 500 AD to present	Keeping <i>A. cerana</i> in purpose-built traditional
4	1930s or earlier to present	Keeping <i>A. cerana</i> in movable-comb hives
5	1876 (or early 1900s) to present	Keeping <i>A. cerana</i> in modern (movable-frame) hives
6	1880s (or early 1900s) to present	Keeping introduced <i>A. mellifera</i> in modern hives

Table 2.2 Rock paintings that provide early evidence of honey hunting. (Crane 1995)

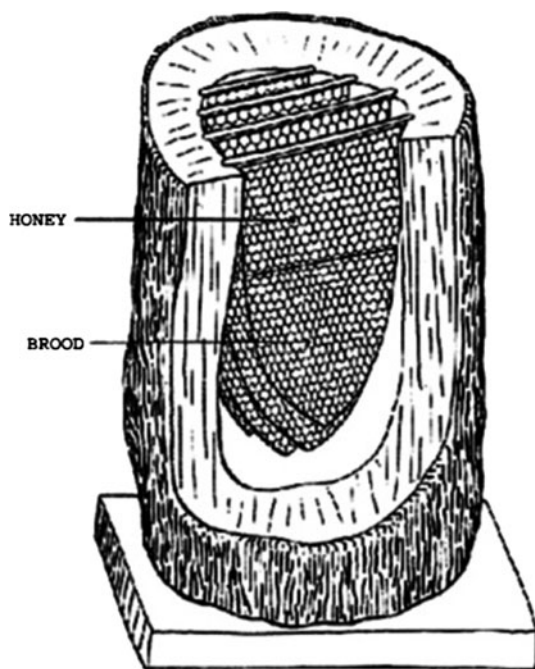
Species of bee	Country	Number	Earliest date known
European <i>A. mellifera</i>	Spain	Several	Mesolithic (6000 BC)
Tropical African <i>A. mellifera</i>	Africa	Very many	Various
<i>A. dorsata</i>	India	Several	Mesolithic
	Sri Lanka	Several	Recent
<i>A. cerana</i>		None known	
<i>A. florea</i>		None known	
Meliponinae (stingless bees)	Australia (Queensland)	One known	Recent

devote a chapter to the subject and quote several cases where a bride's parents gave their new son-in-law a hill or cave containing a number of nests.

Stage 3 is beekeeping proper—the keeping of colonies of bees in hives, i.e. in receptacles that are specially designated or constructed for the purpose; this is essentially an activity of settled agricultural people, in Neolithic times or later. I confine Stage 3 to the use of fixed-comb (traditional) hives. Many early writings mention honey, and some from ancient India and China are several thousand years old. But I would emphasize strongly that a written reference to honey or beeswax does not provide evidence of beekeeping using hives (Stage 3), and neither does a graphic or ornamental representation of a bee. Much honey and wax came from wild colonies, and unless man-made hives are mentioned, or there is some equivalent evidence, we cannot be sure how they were obtained.

It is believed that man has generally obtained honey in the least difficult way available to him—the least hazardous and involving the least number of stings—although honey was so highly prized that he was willing to face both danger and stings to get it. Stage 3, which in most of Asia was beekeeping with *A. cerana*, is therefore likely to have developed earliest where there was no other, more effective, way of getting honey. A wild nest of *A. dorsata* gave a much higher honey yield; thus, on this basis, early beekeeping with *A. cerana* is most likely to have occurred in the parts of Asia that were without *A. dorsata*, including the ecotype *laboriosa*.

Fig. 2.6 Movable-comb log hive used in Vietnam.
(Toumanoff and Nanta 1933)



Stage 4 is the use of movable-comb top-bar hives, such as Sir George Wheler found being used for *A. mellifera* in Greece in 1682 (Crane 1983). In Asia, Toumanoff (1933) and Toumanoff and Nanta (1933) described top-bar hives used for *A. cerana* by some beekeepers in Tonkin in north Vietnam (Fig. 2.6). An upright hollow log was set up with top bars placed across the top, from which the bees built their combs, and photographs show individual combs being lifted out of the hives—the combs were truly movable. It is not clear when or where this type of hive in Vietnam originated, but Mulder (1988) searched for and found some of the hives still in use in the north. Logs had been hollowed out so that the upper half had a larger internal diameter than the lower half, and the top bars rested on the resultant ledge half-way up the hive. The brood chamber thus had movable combs; in the (upper) honey chamber, the bees attached their combs to a board that covered the hive.

Stage 5 is modern beekeeping with movable-frame hives (Langstroth 1853), which had its basis in the Greek top-bar hives found by Sir George Wheler in 1682. The beekeepers in Asia learned movable-frame beekeeping from North America and Europe, where the system was used for *A. mellifera*. However, there is no evidence that it was developed independently in Asia.

There is one other surprise in southeast Asia in addition to the top-bar hives, and this seems to be unique to *A. cerana* beekeeping: tethering the queen, with a hair or a fine thread, in a place where the beekeeper wants a natural or artificial swarm of bees to cluster. The thread is looped around the queen between her thorax and abdomen, using a tie that can finally be pulled undone without touching the queen.

This practice was found in Samui Island, Thailand, and it is described from Burma (Maung 1984) and from Vietnam (Toumanoff and Nanta 1933).

In some parts of Asia, the use of frame hives for *A. cerana* (Stage 5) came after the introduction of *A. mellifera* in frame hives, which have been called Stage 6. In many countries, *A. cerana* and *A. mellifera* beekeeping tends to occur in different regions and, in some, *A. cerana* is still at Stage 3.

Table 2.3 gives, for each country where *A. cerana* is native, the earliest date so far found when beekeeping with *A. cerana* was first recorded: with traditional hives (Stage 3), with movable-comb hives (Stage 4), and with movable-frame hives (Stage 5), also when—if at all—*A. mellifera* was introduced (Stage 6). The countries can be divided into two groups, the first group without *A. dorsata* and the second group with *A. dorsata*, on the grounds that there would have been an early incentive to develop beekeeping (Stage 3) only where *A. cerana* was present, but not *A. dorsata*. Many countries with *A. dorsata* still obtain the greater part of their honey harvest from this bee.

2.13 Regions Where Beekeeping with *A. cerana* is Likely to Have Started Early

2.13.1 *The Northwest: Kashmir, Northern India, Pakistan, Afghanistan*

This region includes parts of the great Indus valley, and especially of the mountain lands that form its upper catchment area (A in Fig. 2.7).

2.13.1.1 Kashmir

In Kashmir, there appears a clear link between beekeeping with *A. cerana* and beekeeping with *A. mellifera* in the Ancient World of the eastern Mediterranean. Horizontal clay hives used in Kashmir were tapered and rounded at one end and were almost identical to hives of which remnants have been excavated at 26 different sites in Greece, the earliest (Fig. 2.8) dated to 400 bc (Crane and Graham 1985). It is astonishing to find that present-day beekeepers in Kashmir pack and sell comb honey in a pair of shallow clay vessels, one inverted on the other to form a lid, as in red pottery hive from about 400 bc, found in the Agora in Athens, Greece (photo from American School of Classical Studies at Athens: Agora Excavations). Such vessels can be seen portrayed in a tomb in Egypt dated to 1450 bc. In the tomb painting (reproduced in Crane 1983), pairs of vessels are being sealed together with mud, and this is done in Kashmir today, 3500 years later.

Crane and Graham (1985) give further information about finds in the eastern Mediterranean, and Shah (1984) has suggested that the diffusion of knowledge about

Table 2.3 Probable start of beekeeping with *A. cerana* in different countries of Asia. The year (AD) of the first record is given for Stage 3, beekeeping with traditional hives; Stage 5, beekeeping with movable-frame hives; Stage 6, introduction of *A. mellifera*. (Modified after Crane 1995)

Area present name	Dates		Stage 6 (<i>A. mellifera</i>)	
	Stage 3 (traditional)	Stage 5 (movable frame)	Stage 6 (<i>A. mellifera</i>)	
<i>Outside the distribution range of A. dorsata</i>				
Western Asia				
Kashmir	1450	Shah 1984	Pre-1931	Neve 1931
Neighbouring states of India, e.g. Himachal Pradesh, Uttar Pradesh	?		1940	Singh 1983
Western and Northern parts of Pakistan	?	?		Early 1960s
Afghanistan	?	?		(1927) Ahmad 1984
	?	?		1977 (1955) Woyke 1984
East Iran	?	?		1961 ?
Northeast Asia				
Japan	(643) 1160	Watanabe 1984?	1876	?
Korea	Pre-643/940	Lee 1981	Early 1900s	Choi 1984
Far eastern USSR	1800s	Crane 1988	? No	1904 Crane 1988
China	500	De-Feng and Wen-Cheng 1981; Mahindre 1983; Galton 1971	Pre-1923 Early 1900s	Hanson 1923; De-Feng and Wen-Cheng 1981; Mahindre 1983
Taiwan	?1700	Chang-Shu-young 1988 (cited by Crane 1995)	1929	Early 1900s Hiratsuka 1920/1921
<i>Within the distribution range of A. dorsata</i>				
India and neighbours				
India (except above)	?	1880s 1883	Joshi et al. 1983; Verma 1983 (cited by Crane 1995)	1880s 1910 Joshi et al. 1983; Mutttoo 1944

Table 2.3 (continued)

Area present name	Dates		Stage 5 (movable frame)			Stage 6 (<i>A. mellifera</i>)	
	Stage 3 (traditional)		1875	Punchihewa 1988 (cited by Crane 1995)	1890	Punchihewa 1988 (cited by Crane 1995)	
Sri Lanka	Pre-1855	Punchihewa 1988 (cited by Crane 1995)	1875	Punchihewa 1988 (cited by Crane 1995)	1890	Punchihewa 1988 (cited by Crane 1995)	
Nepal	?		?1968	Drescher and Crane 1982	? No		
Tibet	?		?		?		
Bhutan	? No		1980	Jorgensen 1983	1988		Baron 1988
Southeast Asia							
Bangladesh	1947	Alam 1983	1965	Alam 1983	? No		
Burma	?		? 1979	Drescher and Crane 1982	? No		
Malaysia	?		1950s	Phoon 1983	1930s		Phoon 1983; Straits Times 1939
Singapore	?		?		1929		Lim Choo Kiat 1954
Indonesia	1864	Patra and Suwanda 1988	1918	Patra and Suwanda 1988; Soekiman 1984	1877		Bee World 1924; Sukartiko 1981
			1920s	Atmosoedaryo 1976	Pre-1924		
Thailand	?		1950s	Akratanakul 1984	(1940) 1950s 1970		Wongsiri 1988 (cited by Crane 1995); Alam 1983; Wongsiri 1988 (cited by Crane 1995)
Kampuchea	? 1950s		?1950s		?		
Laos	?		?		?		
Vietnam	?		Pre-1933 (also stage 3)	Toumanoff 1933	1947		Masse 1947; Mathpal 1984
Philippines	1978	Cadapan 1984 (cited by Crane 1995)	1978	Cadapan 1984 (cited by Crane 1995)	1913		Cadapan 1984 (cited by Crane 1995)

Fig. 2.7 Natural boundaries of *A. mellifera* (.....), *A. cerana* (-----) and *A. dorsata* (.) based on data from Ruttner (1988). The two regions where *A. cerana* is native, but not *A. dorsata*, are marked A and B



Fig. 2.8 Red pottery hive from about 400 BC found in the Agora in the Athens, Greek (photo from American School of Classical Studies at Athens: Agora excavations). (Crane 1995)



beekeeping and honey handling from that region reached Kashmir, where it was applied to *A. cerana*. He points out that in very early times, the ruler and the people of Kashmir were Hindus, and followers of the Hindu religion are not traditionally beekeepers. Between 1420 and 1470, however, Kashmir was ruled by Zain-ul-Abidin, a Muslim who introduced craftsmen from the Middle East, including Egypt, probably via the Indus valley. A first-hand account from 1819 to 1825 by Moorcroft and Trebeck (1940) may well indicate what beekeeping in Kashmir was like soon after its introduction, and his account differs little from traditional beekeeping there today.

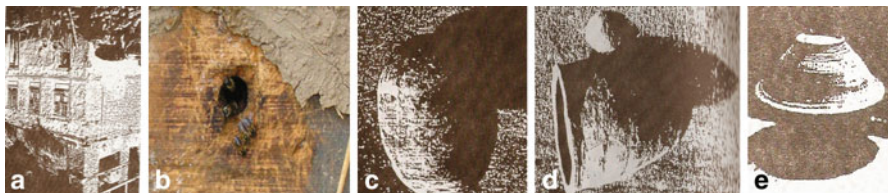


Fig. 2.9 Hive entrance in a house wall in Kashmir (a and b) and red pottery vessel hive in Kashmir (c, d and e). (Crane 1995)

In Ancient times, in both Egypt and Greece, more or less cylindrical hives were placed horizontally, stacked in piles in the open or embedded in specially built walls. Kashmir has a much more severe winter climate, and traditional hives are laid on shelves in the thickness of house walls (Fig. 2.9), any one house having up to 20 or more; a flight hole is provided for each hive through the wall, and honey is harvested from inside the house.

2.13.1.2 Neighbouring Regions

The practice of siting horizontal hives in the thickness of house walls is also well developed in other areas at the head of the Indus valley. In parts of northern India, Singh (1983) describes rectangular wall cavities (*jalas*), with a wooden closure plastered on the inner side, as common in parts of Uttar Pradesh, hollowed logs (*dandas*) being used to provide wall cavities in the higher hills. In the valley of Swat, high above Peshawar in Pakistan, cavities are also left in the house wall (Verhagen 1971): the wooden closure of the hive forms part of the room wall and honey is harvested from inside the room by removing the closure, as in Kashmir. A variant in Nepal is described later. Something similar is done in Ladakh above the valley of Kashmir; outdoor hives there are square, of wood, and honey is harvested from the top of them. In Afghanistan, a hollowed log is sometimes placed inside the length of the house wall. To the west of the Afghanistan border, in Nuristan and in Paktia farther south, cavities in the mud house walls are lined with a wooden framework (Schneider and Djalal 1970; see also Kloft and Kloft 1971; Nogge 1974), or a cooking pot may be laid on its side in the cavity to serve as a hive. The areas mentioned are up to 2,000 m above sea level (Melkania et al. 1983). In parts of Uttar Pradesh, annual honey yields per hive (presumably modern) are quoted as 6–10 kg at 1,000–1,700 m, 10–15 kg at 1,700–2,100 m and 12–16 kg at 2,100–2,700 m. In Turkey, I have seen wall cavities, such as those described, near sea level by the Sea of Marmora, where they are used as hives for *A. mellifera*.

The western boundary of *A. cerana* is separated from *A. mellifera* territory farther west by a belt of desert, shown in Fig. 2.7. According to Gassparian (1977), *A. cerana* bees have been kept in clay pots and gourds for some centuries in Baluchistan (in Pakistan, west of the line joining Quetta and Karachi); hives of these bees were taken from Baluchistan to Iran by man. *A. cerana* has also been recorded in the mountains

of north Khorassan (Pourasghar 1986). More information is needed on this foreign introduction of *A. cerana* into Iran.

2.13.1.3 The Northeast: Japan, Korea, Russian Far East, China

A. cerana is native farther north in the east of Asia (45°N) than in the west (35°N) (Fig. 2.7). In Europe and Africa, where beekeeping with *A. mellifera* developed, most traditional hives were laid horizontally like their prototype in ancient Egypt (which is known from 2500 BC), except in the north of Europe. There, hives stood upright, following the pattern of a log from a tree containing a wild colony, as in tree beekeeping. The dividing line between horizontal and upright hives was roughly the chain of mountains running from the Pyrenees in the west, along the Alps, to the Caucasus and beyond in the east. However, there have been many enclaves of upright hives to the south of this line and of horizontal hives to the north of it.

Traditional hives for *A. cerana* in Asia seem to follow a somewhat similar pattern—horizontal in the south and upright in the north. At present, perhaps because of our less complete knowledge, the pattern appears to be more confused.

2.13.1.4 Japan

According to Ko Watanabe (1984), the earliest record of beekeeping in Japan is dated to the year 643 AD, when Prince Yoha of Kudora in Korea tried to keep bees ‘in 4 combs (hives?) at a holy mountain, Miwayama’, but he failed. Traditional hives in Japan are of various shapes, but mostly with only one chamber. Many are of wood, with an outer protection against the weather. Ochi (1985) described a variety in use in Shikoku Island. Tsushima Island, which lies between Korea and Japan, is an important stronghold of traditional beekeeping with *A. cerana*.

One type of hive used in Kumano, and illustrated in 1795, was different. It was made up of a tier of shallow square boxes (without frames) stacked one above another. The boxes were secured together by a wide cross-bar across the top and the bottom of the hive, and by an upright on each side of it. The combs were continuous from top to bottom of the hive and honey was harvested by slicing through combs between boxes; the top boxes thus served as honey supers. I have seen these tiered hives in museums in Japan. Ko Watanabe (1984) mentioned that the first experimental *A. mellifera* colonies in Japan were brought by his father, Hiroshi Watanabe, to Gifu in 1901; the first colonies used for practical beekeeping came from Hawaii in 1910.

2.13.1.5 Korea

There is a reference to bees in the Kingdom of Koguryo dated to c. 30 BC (Choi 1984) but not to beekeeping. A manuscript from 300 AD refers to the harvesting of honey from wild nests: wood honey came from old tree trunks and stone honey from rocks

(Lee 1981). However, the record from 643 AD, of an attempt by a Korean Prince to keep bees in Japan, suggests that beekeeping in Korea had an early origin, and there is a 940 AD record of Buddhist monks starting to keep bees (Lee 1981). Traditional Korean hives are upright logs, fairly similar to that in the photographs published by Sangyo-Yoh (1934), and Takata (1937) indicated their dimensions and the method of construction. He also mentioned the use (in Zanra-nando Province) of hives built up from sections of hollow log, each 15–18 cm high; they were piled in a tier and added to as the colony grew. It has been said that this type of hive had been brought there some hundreds of years ago by the resident priest, but I do not know the evidence.

2.13.1.6 Russian Far East

The native distribution of *A. cerana* extends north of Korea into the Primor'e (Far Eastern) Province of the USSR. According to the available information, beekeeping did not begin there until the 1800s when peasants were emigrated to this coastal region from southern Russia. The beekeepers among them tried to keep *A. cerana* in log hives, such as they had used at home for *A. mellifera*, but were not successful. Then, in 1904, the first Trans-Siberian railway was completed and subsequent emigrants from Ukraine were able to take with them their own hives of Ukrainian *A. mellifera* and, in due course, modern beekeeping in the region developed successfully. The *A. cerana* population has since declined and, as a result of mite infestation and insecticide poisoning, there are now only a few colonies in the woods (Bilash 1987).

2.13.1.7 China

Beekeeping is said to have begun about 500 AD (De-Feng 1984). Books written after about 1000 AD show that the bees were kept in wooden tubs and bamboo cages (baskets?), and that honey and wax were harvested in late autumn and early spring. Gong (1983) says that by the 1300s, many households were keeping bees. Various types of traditional hives have been used for *A. cerana* (De-Feng 1984). Hollow logs (30–60 cm in diameter and 1 m long) were positioned horizontally or, in the north, stood upright. Wooden tubs—also used in Japan and Korea—were 40–50 cm in diameter at the bottom and 30–40 cm at the top, and one tub might be supered with another. Long rectangular hives of boards were used, sometimes with a honey chamber below. Bamboo and wicker baskets were coated with slurry (probably cow dung and mud) to make them weather-tight.

As in the upper reaches of the Indus valley, a cavity for bees might be built into house walls; dimensions could be about 20–30 cm deep, 40–50 cm wide (along the wall) and 30 cm high. Frame hives were used for *A. cerana* in Manchuria before 1922 (Hanson 1923) and in Guangdong, Fujian, from the 1930s. Meanwhile, in the early 1900s, Caucasian *A. mellifera* in movable-frame hives had been introduced from Russia in Xinjiang (Sinkiang) and other regions. Then, in 1910, Italian *A. mellifera* were imported from Japan, and their use spread rapidly in Beijing, Shanghai and Hopei Provinces.

2.14 Regions Where Beekeeping Is Likely to Have Started Late

2.14.1 *The Rest of India and Neighbouring Countries*

We shall consider first the whole of India (except for the parts adjacent to Kashmir and other areas that drain into the river Indus), together with Sri Lanka, Nepal, Tibet and Bhutan. Muttoo (1944) commented on the surprising absence of references to beekeeping in the vast literature of ancient India, which contains so many mentions of honey. The most likely explanation seems to be that wild nests of *A. dorsata* were the main source of honey then, as they are today. Movable-frame beekeeping was introduced in India during the 1880s (Joshi et al. 1983), but developments were very slow. The All-India Beekeepers' Association was founded in 1937, and the Indian Bee Journal in 1939. Since 1949, the Khadi and Village Industries Commission has organized *A. cerana* beekeeping, first on a local and then on a national level (Thakar 1976). The use of *A. mellifera* is confined to Punjab and some areas farther north.

2.14.2 *Sri Lanka*

To the south of India is Sri Lanka, where pot hives were used at least as early as the 1850s, and frame hives in 1875. *A. mellifera* has been introduced several times but has not succeeded. According to Lanerolle (1984), 65 % of the honey still comes from honey hunting, of both *A. dorsata* and *A. cerana*.

2.14.3 *Nepal*

In Nepal, *A. cerana* colonies are kept in horizontal log hives, hung or otherwise supported under the eaves of the mud houses. This sheltered domestic location is well suited to bees that are gentle; similar hives are placed there, for instance, in Bali, in Indonesia for *A. cerana* and in Central America by Maya Indians for *Melipona beecheii*, one of the stingless bees. Near Pokhara, in western Nepal, I have seen a cavity left for bees in the east gable wall of houses; one climbs up into the roof space of the house to reach the hive, and a wooden shutter is removed to gain access to the combs. It seems likely that this system is derived from the developments at the head, of the Indus valley. Some tribes, such as the Gurung, collect honey from *A. dorsata* (and *laboriosa*) on rock faces north of Pokhara, where honey collectors and beekeepers lived in the same area, with little or no interconnection between them. Modern movable-comb hives with top bars but no frames are also used in Nepal, especially through Gordon Temple's enterprise, and there are a few frame hives.

2.14.4 *Tibet and Bhutan*

Tibetans were officially forbidden to take honey from bees' nests, since their Buddhist religion does not allow them to deprive animals of their food (Harrer 1954). Honey harvesting from *A. dorsata* nests in Tibet has been done by Tibetan people from Nepal. Modern beekeeping with *A. cerana* may by now have been introduced by the Chinese. In Bhutan, a large part of the population is also Buddhist, and there is very little beekeeping. Frame hives of *A. cerana* were brought in from India around 1980 (Jorgensen 1983).

2.14.5 *Southeast Asia*

Last, we come to the countries of southeast Asia: Bangladesh, Burma, Malaysia, Singapore, Indonesia, Thailand, Kampuchea, Laos, Vietnam and the Philippines. Beekeeping is relatively new throughout most of this large region, except possibly in areas to the north that border on China or have been under Chinese influence, but the subject has been little explored. The use of movable-comb top-bar hives in Vietnam as referred in the 'Introduction' seems to have its origin in China. The earliest dates for beekeeping anywhere in southeast Asia are not much more than a hundred years ago, and a few are known to be quite recent (Table 2.3). Two islands where traditional beekeeping with *A. cerana* seems to have flourished earlier than on neighbouring lands are Samui Island in Thailand, where it is likely that Chinese traders introduced it (Wongsiri 1988), and Bali in Indonesia. Statements are often made that beekeeping in a certain country existed 'many centuries ago', 'from earliest times' or 'in the mists of antiquity', but one needs supporting evidence before believing them. Table 2.3 includes the earliest dates as I have been able to ascertain, and I am indebted to participants at the meeting in Malaysia for some of them. In some countries, beekeeping seems not to have started until movable-frame hives were used. Where *A. mellifera* beekeeping has subsequently been introduced into suitable areas, as in China and Japan, for instance, there has been less incentive to proceed with *A. cerana* beekeeping in those areas. There are, nevertheless, large areas where *A. cerana* is the only choice for modern beekeeping.

2.15 *Diagnostic Features of Hives for A. cerana*

This chapter is concerned specifically with the history of beekeeping in Asia with *A. cerana*. We are only at the start of exploring this, and there is a great need for archaeological evidence to support it, and of the type that has been accumulated for beekeeping with *A. mellifera* (Crane 1983). Such evidence should be sought especially in the areas where beekeeping started relatively early, i.e. beyond territory of *A. dorsata*. Searches in other areas may also bring some surprises. Diagnostic

features of hives for *A. cerana* are listed below, adapted from Crane and Graham (1985), and it would be useful if these could be brought to the notice of archaeologists who may be working within *A. cerana* territory, to help them to recognize finds that may be remnants of hives. Asia is, at present, a virgin field for beekeeping archaeology.

A purpose-built hive for *A. cerana* is likely to be characterized by the following.

- a. Its material(s) and construction: Rigid, bee-proof, weather-proof, giving some thermal insulation, of almost any material except metal.
- b. Capacity could be 10–50 L, or even outside these limits: The larger hives would be expected in the north and at high altitudes, where bees are.
- c. Entrance hole(s) for bees, commonly 1–2 cm across (or a slit about 1 cm wide): Entrances may be provided in the hive or in its closure, or by irregularities in construction, especially at the junction of two surfaces, e.g. between a hive and its closure or stand. Hives (and also certain other containers) may have small holes for ventilation or for cord used to secure two parts together.
- d. Means of access by the beekeeper when removing honey combs: This can be provided for by using an open-bottomed hive standing on a base (lifting it up to take the combs); by cutting off the flat top of the hive (to which honeycombs were attached); or by incorporating a large, removable closure at one or both ends of a horizontal hive, or on one side of an upright hive. Alternatively, some primitive hives are made of disposable material such as mud (with or without animal dung). They are broken into to reach the honey combs and immediately repaired by applying more mud.
- e. Support for the combs: Bees build their combs down from the top of their hive, so a hive must have a solid top. A horizontal hive may show combing (in pottery) or parallel grooves (in hewn wood) on the inner roof or upper part of the inner walls. The purpose of such treatment is to provide good attachment for the bees' combs and/or to persuade the bees to build their combs in a certain direction. A hive used open at the top must have top bars or similar provision to support the combs.
- f. Pottery hives could retain vestiges of beeswax on the inner walls, where combs were attached to them, and confirmation of beeswax, e.g. by chromatography, has proved a valuable diagnostic aid (Graham 1975).

2.16 Conclusion

In conclusion, we should apply our present knowledge of the history of *A. cerana* beekeeping when seeking answers to the following questions: (1) Where is it most beneficial to promote *A. cerana* beekeeping? (2) Where should the use of movable-frame hives be taught and extended? and (3) Should the use of movable-comb top-bar hives be promoted and, if so, where? These hives have not proved as popular for *A. cerana* as they have in some parts of the world for *A. mellifera*, but the reasons are not yet clearly assessed. Where are the benefits of using *A. mellifera* likely to be so

great that, they should be weighed up against the dangers associated with importing this bee? These include the introduction of diseases and parasites, competition with and possible extinction of beekeeping with *A. cerana* and of *A. cerana* itself, and associated ecological changes. Finally, there are many areas where both *A. cerana* and *A. dorsata* are native, and the major honey production is still by collection from wild nests of *A. dorsata*. In which of these areas is the best aim for research and extension of the extended use of *A. cerana* in hives? In which areas would it be better to concentrate on upgrading honey harvesting from *A. dorsata*—either unmanaged in the wild or managed, for instance, in the way developed in India (Mahindre 1983; Crane 1990). This would largely complete a feasibility study on the exploitation of Asia's honeybee resources.

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Chapter 3

Biology of *Apis cerana*

3.1 Introduction

In social insect societies, only some of the individuals reproduce. Food resources are allocated to either adult workers or sexual offspring (Seeley 1985; Hölldobler and Wilson 1990). In colonies of the highly eusocial honeybees (Apini), drones and virgin queens are produced as sexuals. Colonies should allocate their resources in such a way that the chances for sexuals to contribute to the next generation are the highest. This contribution differs between drones and queens. Drones only need to search a virgin queen during her nuptial flight to mate with, whereas queens need a colony of workers to become successful (Velthuis 1990). Therefore, the production of a successful young queen implies the production of a swarm or supersedure, where the old queen leaves with part of the bees and a young queen takes over the original colony. The size of the swarm and the moment it leaves the mother colony are important factors determining later success of the swarm and of the original colony. Hence, the number of successful young queens that a colony can produce is limited. In contrast, the number of drones produced can be very large because production costs are low and the more drones a colony can produce, the greater is the possibility that some of them will mate with a virgin queen (Velthuis 1990). To promote outbreeding, honeybees have developed a mating system in which insemination of the virgin queen occurs at drone congregation areas far from the colony, where drones from widely dispersed colonies congregate (Fletcher and Ross 1985; Puchihiwewa et al. 1990; Koeniger and Koeniger 2000). In Western honeybees, *Apis mellifera*, the production of drones and queens is governed by a combination of external and internal factors, which has been comprehensively studied (Winston and Taylor 1980; Ruttner 1985; Winston 1987). Much is known from *A. mellifera* colonies in temperate regions where flowering is present during a limited period of the year, which results in a defined seasonal appearance of sexuals and colony multiplication (Seeley 1985; Winston 1987). In contrast, tropical florescence is usually available over the entire year, potentially allowing the colonies to produce sexuals year-around (Allsopp and Hepburn 1997; Hepburn and Radloff 1998).

The number of sexuals produced and the number of swarms issued mainly depends on food resource availability and the number of workers (Winston and Taylor 1980;

Winston 1987, 1990; Allsopp and Hepburn 1997). Moreover, other factors such as fecundity and age of the queen and genetic disposition are involved (Ruttner 1983, 1985). Mechanisms and factors regulating the production of drones and queens may be similar (Allen 1958; Winston 1987).

Currently, the honeybees (Apini) are considered to have nine species (Otis 1997) that form a monophyletic group (Ruttner 1988). Of these, *A. mellifera*, *Apis cerana* (Ruttner 1988), *A. koschevnikovi* (Tingek et al. 1988), *A. nigrocincta* (Hadisoesilo and Otis 1996) and *A. nuluensis* (Tingek et al. 1996) form the cavity-nesting group of *Apis*. Bees in this group are probably rather similar in colony organisation (Dyer and Seeley 1991). On the basis of morphometric variation and geographic distribution, the Asiatic honeybee species, *A. cerana*, has also been classified into four subspecies: *A. cerana cerana* in northern Asia; *A. cerana indica* in southern Asia; *A. cerana japonica* in Japan; and *A. cerana himalayana* in the Himalayan region (Ruttner 1988). Beekeeping with *A. cerana* is an ancient activity. In many natural and agricultural ecosystems, *A. cerana* is the most important pollinator (Verma 1990; Crane 1991; Kevan 1995). Since this species is distributed over a wide range of natural habitats and climates, in a vast geographic area of Asia ranging from Iran to China and from Japan to the south of Indonesia (Smith and Hagen 1996), *A. cerana* would be expected to express basic differences in colony growth and reproduction in response to environmental differences. For example, the reproductive season is confined to 3 months in spring in the temperate region of Japan (Okada 1970; Matsuura and Sakagami 1973). In the tropical monsoon region of northern Thailand, southern India and northern Pakistan, colony reproduction usually occurs from March to October (Koeniger 1976; Seeley et al. 1982; Seeley 1985; Ruttner 1988; Roubik 1989) when no seasonality in colony reproduction has been found in the tropical rain forest of Sumatra (Inoue et al. 1990).

Although reproduction has been widely studied in *A. mellifera*, relatively little work has been done on the sister species *A. cerana* (Koeniger and Koeniger 1991). For *A. cerana*, much of available data focuses on mating behaviour, physiology and reproductive isolation (Koeniger and Koeniger 2000). Little information exists on seasonal cycles of colonial development, production of sexuals, swarming and supersedure. Mechanisms and factors regulating these processes are important not only for insight into the evolution of these social bees but also for application in the breeding programs of the Asiatic honeybees in many Asian countries (Verma 1990).

Before continuing the description and analysis of *A. cerana* from existing studies, a caveat should be introduced at this point. Care should be taken when interpreting aspects of the biology and ecology of *A. cerana* reported in the literature. This is simply because what is known about the bee comes from particular places and times. That information clearly shows that *A. cerana*, like *A. mellifera*, exhibits a great deal of plasticity in its biology and ecology across its geographical range. Thus, the biology and ecology attributed to *A. cerana* at one location in Asia does not mean that those attributes will apply to *A. cerana* at other locations. It also does not prove that current or future *A. cerana* genotypes that arrive in Australia will show general *A. cerana* traits in Australian environments.

It has been claimed that *A. cerana* (also known as Eastern honeybee, Asiatic hive bee, Mee Bee) is 'the exact equivalent, in the Eastern part of the Old World, of its occidental sister species *A. mellifera*. It has an equally wide area of distribution with a similar capacity for a broad spectrum of adaptations' (Ruttner 1988). However, although a honey producer, it does not hoard large quantities of honey and it is therefore not the preferred species for honey production, compared with *A. mellifera* (Koenier et al. 2010). It is one of the species of social honeybees which demonstrate both individual and colony behaviour—a key to maximising invasion potential. Such flexibility enables them to withstand biotic resistance and to better match conditions in the receiving community (Moller 1996).

Chinh et al. (2005) in their study found that in northern Vietnam, observations on colony growth, on production of drones and queens and on swarming and supersedure in *A. cerana* were related to available flowers to forage on and climatic data. Despite the tropical setting of the study area with forage available year-round, production of sexuals (i.e. drones and queens) was restricted to two periods from March to July and from September to December. Most swarming occurred in May when forage was most abundant.

Mohapatra and Satapathy (2012) studied the colony developmental characteristics of Indian honeybee *A. cerana indica* in Bhubaneswar, Orissa, India, and found that the brood area, number of frames with eggs and pollen store were maximum during December and were positively correlated. Similarly maximum bee population was recorded during May coinciding with maximum honey store showing a positive correlation. Nectar gatherers and pollen gatherers were maximum in January and February, respectively. Dearth period was observed to be during August and September. Das and Rahman (2000) studied the brood rearing activity of *A. cerana indica* in terms of egg-laying capacity of queen, brood area and brood population. They found that maximum egg laying was 673 eggs per day in March and the minimum 28 eggs per day in August, with an annual average of 276 eggs per day. The maximum brood area was recorded to be $1,924.25 \pm 196.37 \text{ cm}^2$ in February and the minimum $258.85 \pm 41.53 \text{ cm}^2$ in August. Similarly, the highest brood population was enumerated to be $10,960 \pm 1,090$ during February and the lowest $1,430 \pm 230$ in August. Brood population of *A. cerana indica* workers exhibited a significant, negative and linear correlation with minimum and maximum temperature, relative humidity and rainfall; however, a positive correlation was observed with bright sunshine hours.

Production of sexuals and swarming in *A. cerana* was strongly synchronised in two periods of the year, of which the period from March to June was the most important by far (Chinh et al. 2005). The synchronised production of sexual offspring has also been found for *A. cerana* in other regions, both temperate (Okada 1970; Masuura and Sakagami 1973) and tropical (Sharma 1969; Seeley et al. 1982; Seeley 1985; Ruttner 1988; Roubik 1989). In addition, the pattern is rather similar to what has been described for *A. mellifera* both in temperate (Seeley 1985; Winston 1987) and tropical regions (McNally and Schneider 1994; Hepburn and Radloff 1995; Otis 1990; Allsopp and Hepburn 1997; Hepburn and Radloff 1998). The correlations between flowering intensity, colony growth and production of drones and queens suggest that the synchronised production of drones and queens is defined by the

forage flow into the colony. If the flow is high, the colony starts growing, then drones and queens are produced and eventually the colony swarms. If foraging conditions were good during much longer periods, honeybees would swarm more often (up to 12 swarms and after swarms per year) and the synchronised production of sexuals would be lost (Winston 1990; Otis 1990).

Clinch et al. (2005) found no correlation between the production of sexuals and the amount of nectar and pollen stored inside the colony, showing that the bees react directly on forage availability. Under temperate conditions, this link between available forage and production of sexuals may be weaker than under tropical conditions, because at the end of the active season, colonies have to store large amounts of food to survive the long winter.

A. mellifera and *A. cerana* are sister species with similar traits of social structure and behaviour (Seeley 1985; Dyer and Seeley 1991). Both species have evolved to exploit flower patches for maintaining their colonies and subsequently to invest as much as possible in production of sexuals (Seeley 1985; Velthuis 1990). Therefore, it is not surprising that the general pattern of seasonality in colony growth and production of sexuals is similar between *A. cerana* and *A. mellifera*. Variation may result from the different environment the bees live in, rather than from fundamental differences between the species. For example, in *A. mellifera*, temperate-evolved bees invest a lower proportion of drone comb at relatively small colony sizes than tropical evolved bees, but more drone comb in larger colonies (Lee and Winston 1985; Winston 1987; Winston 1990).

The colony size affects production of sexuals in *A. cerana* (Inoue et al. 1990) as well as in *A. mellifera* (Lee and Winston 1985; Winston 1990; Allsopp and Hepburn 1997). Colonies initiated drone and queen rearing when their population was larger than 10,000 bees, which is similar to earlier studies of *A. cerana indica* (Sharma 1969; Inoue et al. 1990) and *A. mellifera* (Free and Williams 1975; Lee and Winston 1985).

3.2 Colony Multiplication in Honeybees Takes Place Through Swarming

Queens are produced in a small number and appropriate to the needs of the colony, while 100–1,000 times more drones are produced. This ratio probably reflects the relative costs a colony has to invest to get equal fitness returns. The efficiency of drone production defined as the chance for a drone to mate seems to vary much throughout the year; however, most of the drones produced in the winter period are chased away from the hives by workers and die soon after their production. In addition, the number of young queens produced is also very low. Why do the colonies produce so many drones when they are killed shortly afterwards and when very few virgin queens are available to mate with in winter time? Unfavourable conditions after the start of drone rearing apparently make production inefficient in winter. It

indicates that rearing of drones is the expression of a certain surplus capacity in brood rearing (Velthuis 1990), and immediately responds to resource availability.

In both species, bees seem to respond quickly to changes in floral resources by adjusting the extent of brood rearing. This affects colony growth, which in turn affects production of sexuals and eventually swarming. Because during large parts of the year floral resources are often not sufficient to warrant colony growth, seasonal patterns in the production of sexuals appear.

The true honeybees (the genus *Apis*) are a biologically well-defined group within the family Apidae which comprises, besides *Apis*, the Euglossini (orchid bees), Bombini (bumble bees) and Meliponini (stingless bees). It is important to realise that all groups of Apinae share many traits, which they have inherited from their common ancestor. Nesting in a cavity seems to be a good example of an ancestral feature which most Apidae have retained. According to Winston and Michener (1979), relation between the Meliponini and *Apis* is not as close as once thought. Many of their common characteristics, like the forming of perennial colonies and division of labour, have apparently arisen independently.

The main characteristics which separate *Apis* from all other bees are listed as follows:

1. The comb is constructed out of pure bee's wax. No other material is added as in bumble bees or Meliponini.
2. The construction of a vertical comb with horizontal hexagonal cells, which open to both sides and the repeated use of these comb cells for brood rearing and storage of pollen and honey.
3. The use of 9-oxo-2-decenoic acid as a main component of the queen's pheromone and as a major component of the queen's sexual attractant during mating.
4. The use of isopentyl acetate as the main alarm pheromone.
5. The dance communication which serves to direct colony members to newly found food sources.
6. Colony multiplication by swarming, which is initiated by the sudden departure of the old queen together with a large number of workers.

This list represents only a small number of the many common features in *Apis*. All traits, taken together, leave no doubt that all honeybees are closely related and have had a long common evolutionary path after separating from the other Apinae.

Each species is highly variable with a broad range of polymorphism which was, and is, subject to many attempts of reclassification. Intensive and careful biometrical and biogeographical analysis of *A. mellifera* (Ruttner 1988) shows that parameters of body size, hairs and colours vary widely. Many extremely different races of *A. mellifera* have been crossed and fertile hybrids were obtained. Thus, it is prudent to base decisions on recognition of species on characters of the reproductive and genital organs. In the case of the nine species of *Apis*, the drones have clear species-specific structured endophalli (Fig. 3.1). As judged by genital characters, the large free-nesting bee of the Himalayan region, originally described by Sakagami et al. (1980), should be regarded as a subspecies named *A. dorsata laboriosa*.

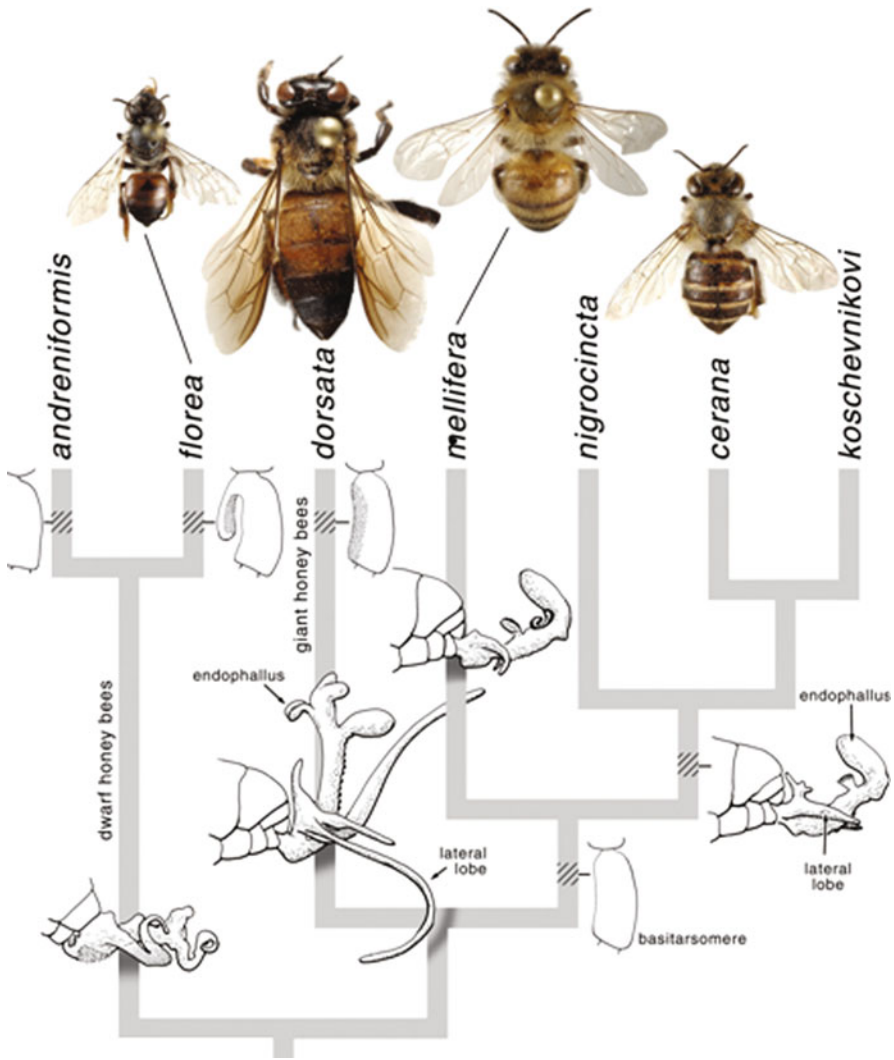


Fig. 3.1 The male copulatory organ is a complex endophallus in *Apis*. The differences among the species are significant. Relationships among species of recent honeybees, genus *Apis*, showing important variations in tarsomeres and male genitalia. Relationships from Engel and Schultz (1997)

The ‘principal’ difference among the honeybee species is the mode of nesting. It has an important impact on the colony as well as on the life of the single bee. Thus, the genus *Apis* can be divided into two subunits: the open-nesting honeybees, *A. florea* and *A. dorsata*, and the cavity-nesting species, *A. cerana* and *A. mellifera*. The open-nesting species normally build only a single comb per colony, but both species show several distinct differences in their nest construction. *A. florea* builds its comb

around a small branch or other kinds of supports in such a way that the comb has an open convex surface on the top. *A. dorsata* constructs its larger comb under a rock or large branch, so the upper portion of the comb is never exposed.

The hive bees, *A. mellifera* and *A. cerana*, do not show any major differences in nest construction. Both species nest in cavities where they build several parallel combs. The combs are built under the ceiling of the cavity and attached to the cavity's walls. The arrangements and distribution on the combs is similar in all races of each species. In the wild, *A. cerana* prefer to nest in small spaces, such as hollowed out tree trunks. Like the Western honeybee, they are also domesticated and used in apiculture, mostly in wooden boxes with fixed frames. Their size is similar or somewhat smaller than *A. mellifera*, and they also have a more prominent abdominal stripes. These bees can be adapted to living in cavities in some human structures and in purpose-made hives, and their nesting habit means that they can potentially colonise temperate or mountain areas with prolonged winters or cold temperatures. Honey is stored in the upper portion of the comb. In the central and lower part the comb, cells are used for brood rearing. Pollen storage cells are found between the brood-rearing area and the honey cells. Queen cells are constructed at the lower edge of the

3.3 Flight Patterns and Swarming

A. cerana bee flights are reported to be similar to fly flights in that they are rapid and unpredictable compared with *A. mellifera* flights patterns. There is also some unpublished evidence that *A. cerana* colonies in hives demonstrated 5.5 times as much flight activity relative to the number of bees in a colony compared with *A. mellifera* (Ruttner 1988, p. 138). However, they tend not to fly far from their nests to forage; one source claims that this distance can be as far as 750 m but that 300 m is more typical (Punchihewa 1994). Swarming activity associated with *A. cerana* reproduction is reported differently in different countries. In Japan, Tokuda recorded one, two or three swarms per colony per year, while researchers in Pakistan recorded an average of eight swarms per year (cited in Ruttner 1988). Koeniger et al. (2010) report that in tropical conditions, swarms can survive and travel for several weeks; however, longer periods of nectar scarcity or extended periods of rain will put the survival of a swarm at risk. In Taiwan, Fen Tsung Deh reported regular seasonal migration swarms by *A. cerana* between humid mountain areas and flatter areas (Fen Tsung Deh 1952; Ruttner 1988). In Australia, extensive field observation of the limited incursions to date indicate that there may be a difference in swarming behaviour depending on whether they are (a) reproductive swarms (1–2 per year) or (b) absconding swarms (up to 7 per year). There may also be a difference in swarming behaviour depending on whether *A. cerana* is in colonisation ('bunker down after moving in') or invasion mode ('up stakes and spreading out'). Swarms of *A. cerana* which abscond generally do so in response to a shortage in floral resources, an attack or approach by predator/s or disease outbreak, e.g., from wax moths. Absconding behaviour is reported differently in different countries, with more frequent reports of absconces from Thailand and in temperate Japan and less frequent reports in south Asia (Ruttner 1988).

3.4 Natural Distribution

A. cerana, or the Asiatic honeybee, is a small honeybee found in southern and southeastern Asia, including all the countries of the Himalayan region (Afghanistan, Bangladesh, Bhutan, China, India, Myanmar, Nepal, Pakistan) as well as Indonesia, Japan, Malaysia, Papua New Guinea, Thailand, Vietnam and probably other countries. This species is also known as the Himalayan hive honeybee. This species is a sister species of *Apis koschevnikovi*, and both are in the same subgenus as the Western (European) honeybee, *A. mellifera*.

The natural distribution of the true honeybees is restricted to the old world (Asia, Europe and Africa). *A. mellifera* was imported by immigrants from Europe to Australia and the Americas.

The natural distribution of *A. mellifera* is restricted to Africa and Europe. In many parts of Asia, all of the other three species, *A. florea*, *A. dorsata* and *A. cerana*, share their habitats. Naturally, both species of hive bees are allopatric—meaning their territories are geographically isolated from each other. To the west, *A. cerana* is found as far as central Afghanistan. The eastern limit of the range of *A. mellifera* is Mashad in Iran (Ruttner et al. 1985). They are isolated from each other by more than 500 km of desert in which probably no bee colonies exist. In the north, *A. cerana* is found up to 46° latitude (Ussuria). The eastern territories of *A. cerana* include the Japanese islands (with the exception of Hokaido), Philippines, Celebes and Timor. Wallace's line represents the eastern border in the south. No natural overlapping or direct contact between *A. cerana* and *A. mellifera* is known. Reports or speculations of the existence of bees transitional between *A. cerana* and *A. mellifera* (Deodikar and Thakar 1966; Shah 1980) are not supported by studies on their natural distributions.

The introduction of *A. mellifera* to Japan reduced the local *A. cerana* population to the point where '*A. cerana* is found only in remote mountainous areas, almost like a relic which could soon become an endangered species' (Ruttner 1988, p. 161). However, just the opposite occurred in parts of the Solomon Islands where the introduction of *A. cerana* led to the total extinction of the exotic *A. mellifera* populations on some islands (Anderson and Annand 2010).

On average, *A. cerana* are smaller than *A. mellifera*. *A. cerana* is the third smallest of the nine species of honeybee (Koeniger et al. 2010). However, it should be noted that there are some genotypes of *A. mellifera* that are smaller than medium-sized *A. cerana* genotypes and there is no significant difference between the smallest genotype of both species (Ruttner 1988). However, the bigger *A. mellifera* genotypes considerably outsize even the largest *A. cerana* genotypes. *A. cerana* has distinctive stripes on its body compared with its European cousin *A. mellifera*. Figure 3.2 gives some indication of the relative size and appearance of each species.



Fig. 3.2 Four species of *Apis* (from left to right: *A. florea*, *A. dorsata*, *A. cerana* and *A. mellifera*). *A. florea* colony builds its comb around branches. The comb is covered by a layer of bees. *A. dorsata* colony builds its comb under a thick branch. The bees cover the comb totally



Fig. 3.2 (continued)

3.5 Nesting

As a species of cavity-dwelling bees, *A. cerana* colonies nest in hollow trees, caves, rock clefts, walls roof spaces and rafters as well as cavities provided by birds, small mammals and tree-dwelling reptile species. *A. cerana* occupies smaller hives than *A. mellifera* because of their smaller physical and colony size, and there is evidence that *A. cerana* colonies fail in standard size hives (Pandey 1977 cited in Ruttner 1988). However, in natural environments *A. cerana* build nests in cavities with volumes as small as 4.5 L to as large as 97 L (Inoue et al. 1990 cited in Olroyd and Wongsiri 2006). In one study, *A. cerana* was found in a tree cavity with a diameter of 12 cm

(Inoue et al. 1990 cited in Olroyd and Wongsiri 2006, p. 153). Unlike other cavity-nesting bees, cavity entrances of *A. cerana* nests vary widely (from 2 to 100 cm²) and are often found within 1–2 m off ground level (Inoue et al. 1990; Seeley et al. 1982 cited in Olroyd and Wongsiri 2006).

A. cerana are one of the cavity-nesting species which thermoregulate nest temperatures. Where external ambient temperatures may vary between 12 °C and 36 °C, this bee species is able to maintain the brood nest temperature in the range of 33–35.5 °C. In particularly hot weather, *A. cerana* will use evaporative cooling mechanisms, collect water and cluster outside the nest. In particularly cold weather, *A. cerana* has been observed to use metabolic heat to warm brood nests. There is some evidence (well documented in Ruttner 1988) of *A. cerana* routinely dismantling old combs in nests in order to build new cells upon it. Arguably, this may contribute to more hygienic practices at the comb site, but less as the old wax debris accumulates on the bottom of the hive and provides a suitable medium for wax moths (Ruttner 1988).

Species of *A. cerana*, *A. dorsata*, *A. laboriosa*, *A. florea* and *A. andreniformis* are the primary host of three different genera of mites, *Tropilaelaps*, *Euvarroa* and *Varroa*. Mites in the genus *Tropilaelaps* are parasites of the giant honeybees of Asia (*A. dorsata* and *A. laboriosa*) (Anderson and Morgan 2007). Some are occasionally observed inside *A. cerana* colonies in Asia (Ruttner 1988; Otis and Kralj 2001). However, except for one rare instance in Asia (Anderson and Morgan 2007) there is no other evidence that these reproduce on *A. cerana* brood (Otis and Kralj 2001). Mites in the *Euvarroa* genus are hosted by *A. florea* and *A. andreniformis*.

A. cerana is host to three different kinds of *Varroa* mites, including *V. jacobsoni*, *V. underwoodi* and *V. destructor* depending on the genotype of *A. cerana* (Anderson and Trueman 2000). So far, only genotypes of *A. cerana* from northeast mainland Asia and the Japan region carry the forms of *V. destructor* which are extremely damaging to *A. mellifera* globally. The Java genotype of *A. cerana* carries mites that have long been known to be harmless to *A. mellifera*. However, in 2008, a harmful form of the mite was detected in Papua New Guinea (Anderson 2008). This mite did not accompany the Java genotype of *A. cerana* into the Solomon Islands (Anderson and Annand 2010). The bee in the Solomon Islands carries a harmless form of the Java genotype of *V. jacobsoni*. *A. cerana* can effectively remove *V. jacobsoni* through grooming behaviour consisting of self-cleaning, grooming dance, nestmate cleaning, and group cleaning. *A. cerana* worker bees can also rapidly and effectively remove *V. jacobsoni* mites from the brood. However, *A. mellifera* does not demonstrate such a high level of activity.

Like the Western honeybee, *A. cerana* is kept by farmers for honey production and pollination. Traditionally, the bees were kept in log hives, now being replaced by wooden boxes with fixed frames. The *A. cerana* bee size is similar or somewhat smaller than *A. mellifera*, and they also have more prominent abdominal stripes. Their honey yield is smaller, because they form smaller colonies and partly because they have yet to benefit from the in most areas the selective breeding programs that have produced modern day *A. mellifera*. In folk medicine, their beeswax is used to treat and heal wounds. *A. cerana* is found at altitudes from sea level up to 3,500 m in areas with appropriate flora and climate. This bee species has adapted to

adverse climatic conditions and can survive extreme fluctuations in temperature and long periods of rainfall. It is unique in its ability to survive temperatures as low as -0.1°C , a temperature lethal for other bee species (*A. mellifera*).

3.6 Biology

The life cycle of Asian bees (*A. cerana*) is very similar to that of honeybees (*A. mellifera*). Brood is reared for 21 days from egg to hatching adult. The colony is structured in a similar way with a single fertile female (the queen), several thousand worker bees (sterile females) and seasonally male bees (drones). Drone brood cappings have a characteristic pin hole in the middle. The colour of Asian bees can vary considerably, similar to that of the honeybee. Asian bees have a very distinct stripy abdomen. The overall size of Asian bees is approximately two-thirds the size of honeybees. Foraging range is said to be a lot more restricted with Asian bees, with most of the activity occurring within 300 m of the colony and occasionally up to 1 to 2 km flight range, while foraging ranges for honeybees are considerably greater, with 3–4 km common. Colony population size is a major distinguishing feature between Asian bees and honeybees. Asian bee colonies with a population of 10,000 are considered large colonies and population sizes of 2,000–5,000 are reasonably common, whereas honeybee populations usually range between 30,000 and 60,000 and a colony of 5,000–10,000 bees would be considered a nucleus colony. Swarming activity is extreme in Asian bees when compared with honeybees. Asian bees are said to swarm for a range of reasons other than just for reproduction. Absconding swarms and migrating swarms are common and are induced for a range of reasons including a shortage of food, disturbance or pest pressure (ants, wax moth). Swarming distances have been reported up to 10 km from the original colony. Asian bees (*A. cerana*) are cavity-nesting bees which permits them the opportunity to increase their chances of survival in cooler climates. They also appear to favour areas of human habitation, preferring nesting sites 1–2 m off the ground. Densities of Asian bees will vary according to nesting site availability and feed. High densities of colonies have been reported with up to $22/\text{km}^2$.

The European species *A. mellifera* has a natural distribution including the African continent, Mediterranean, Northern Europe and Eastern Europe. *A. mellifera* has been introduced to all the continents except Antarctica. There are over 150 subspecies named within this species, of which only a few have been widely propagated for beekeeping. Asian bees (*A. cerana*) are the next most common *Apis* species around the world. Although it is recognised that there are possibly many subspecies of this bee, only a few are commonly referred to, *A. cerana indica*, *A. cerana japonica*, *A. cerana cerana* and *A. cerana johnei*. *A. cerana javana* is the strain of Asian bee currently in Papua New Guinea and northern Australia.

3.7 Differences Between *A. mellifera* and *A. cerana*

Many morphological and biological characteristics show similarity between both species. A good example of this is the body size which can be precisely measured by the length of the forewing. The range of *A. mellifera* is 9.52–7.64 mm and of *A. cerana* is 8.89–7.47 mm (Ruttner 1988). The smaller races of *A. mellifera*, like *A. mellifera yemenitica*, are smaller compared with the larger races of *A. cerana*. It is important to realise that both honeybee species are polymorphic, and therefore, differences found between one local population of *A. cerana* and another local population of *A. mellifera* reflect regional adaptations to climatic or environmental conditions rather than species-specific differences. This polymorphism has led to many misinterpretations. Historically, many of the earlier descriptions of *A. cerana* were based on subtropical or tropical populations described by investigators who were familiar with populations of European *A. mellifera*. In consequence, many differences listed are not species-specific but are mainly differences between southern and northern honeybees. On the other hand, scientists who were familiar with *A. cerana* classified African populations of *A. mellifera* from Cameroon and Senegal as subspecies of *A. cerana* (Buttel-Reepen 1906; Maa 1953).

Nevertheless, there are useful diagnostic differences between *A. cerana* and *A. mellifera*:

1. The tomentum on the sixth sternite is only found in workers of *A. cerana*.
2. The radial vein in the hindwing of *A. cerana* has an extension which is missing in *A. mellifera*.
3. The endophallus of drones of *A. mellifera* carries chitinous plates which are not found in the endophallus of *A. cerana*.
4. The cover of the capped drone brood cells has a pore only in *A. cerana*.
5. The position of the fanning bee is opposite in each species. In *A. mellifera*, the fanning bee's head is directed towards the hive entrance; in *A. cerana*, the end of the abdomen points to the entrance.
6. *A. cerana* has a specific 'hissing' behaviour which is absent in *A. mellifera*. Colonies of *A. cerana* react to knocking on the hive or comb by producing a hissing sound (for details, see Sect. 3.13).

3.8 *A. cerana* and *A. mellifera* are Well-Defined Species

Because of the overlapping variation of many morphological and behavioural features, the question of whether *A. cerana* and *A. mellifera* are separate species, in the sense of strict reproductive isolation, has required research into their reproduction. Initially it was demonstrated that the drones of *A. cerana* were visiting the same drone congregation areas as those of *A. mellifera* (Ruttner 1973). Indeed, drones of *A. cerana* did follow queens of *A. mellifera*. Drones of both *A. mellifera* and *A. cerana* react to 9-oxo-decenoic acid (Ruttner and Kaissling 1968) which is the sexual

attractant emitted by queens of all *Apis* species (Shearer et al. 1976). Whether an interspecific copulation takes place regularly in drone congregations is not yet known. However, with instrumental insemination, interspecific sperm transfer is possible. Heterospecific sperm implanted into the queens' spermathecae survived for some time. Further, the sperm were seen to move out of the spermatheca to the egg, but the fertilised egg then died (Ruttner and Maul 1983). In conclusion, these experiments suggest that a genetic incompatibility between *A. cerana* and *A. mellifera* exists, and therefore, in spite of all other similarities, *A. cerana* and *A. mellifera* must be recognised as well-defined species.

3.9 Division of Labour

Although division of labour has been extensively studied in *A. mellifera* (Rosch 1927; Lindauer 1952; Seeley 1985), we have little information on this very important aspect of social life in *A. cerana*. In Oberursel, some observations (Perk 1973) were carried out on a colony of *A. cerana* originating from Peshawar (North-West Frontier Province, Pakistan). A group of young bees (2–4 h after emergence) was marked by small numbered tags on the thorax. One of these bees was observed during the following period of 15 days. During the first days, the bee spent up to 60 % of the observation time motionless in empty comb cells. Its other activities were patrolling and cleaning behaviour. On the third day, the bee started to feed larvae. That was its main activity until seventh and eighth day when the bee joined others in comb building. Its first orientation flights were observed on the seventh day. After 15 days, the bee began to forage for nectar and pollen outside. This bee did not act as a guard. Throughout the observation period of 140 h, the bee spent most time patrolling (48 %) and resting motionless on the comb or inside a cell (38 %). Some shorter activities such as food exchange, preparation of pollen and fanning were also observed. A significantly different activity from the typical activities of *A. mellifera* was the large amount of grooming activity which occupied 2.75 h (2 % of the observation time). For half of this time, the bee was groomed by other bees. A special behaviour was noted in which the bee would ask to be groomed.

These observations were supplemented by measurements of the hypopharyngeal glands. The diameters of the acini showed an increase during the first few days, but after the 15th day, there was a decrease. The development of the wax glands was measured by preparations of histological sections by D. Mautz. The maximal development was on days 12 and 13. After day 22, the preparations showed degenerated gland cells. Altogether, the development of the gland systems corresponded well with the behavioural changes. These preliminary observations indicate a similarity between division of labour in *A. mellifera* and *A. cerana*. However, a more detailed study in the natural habitat of *A. cerana* may very well result in important quantitative differences.

3.10 Ecology and Biology

3.10.1 Competition and Floral Resources

There is relatively little written about the floral preferences of *A. cerana*. In 1958, Miyamoto noted that *A. cerana* in Japan visited a wider variety of plant species, including natives, compared with the limited floral preferences of *A. mellifera* (cited in Ruttner 1988). In a 1968 German study of honey from *A. cerana* and *A. mellifera*, clear differences in the pollen spectrum were collected by the two species despite them operating at the same time and in the same vicinity (cited in Ruttner 1988). Some studies claim that *A. mellifera* is clearly dominant over *A. cerana* in common feeding locations (Sakagami 1959; Dhaliwal and Atwal 1970 cited in Ruttner 1988). Another, more recent study suggests the opposite. In the Solomon Islands, substantial losses of exotic *A. mellifera* honeybees were attributed to *A. cerana* robbing *A. mellifera* hives and increasing competition for floral resources (Anderson and Annand 2010). More widely accepted is that *A. cerana* does well in disturbed or extensively modified habitats. For example, in Hong Kong, *A. cerana* visits 86 % of plant species and pollinators so successfully as to maintain that islands diverse flora (Corlett 2001 cited in Oldroyd and Wongsiri 2006). Of relevance to Australian environments, the indica genotype of *A. cerana* in Sri Lanka was observed foraging on *Eucalyptus robusta* (Myrtaceae) (River red gum) and it is cited as being an excellent source of honey (Punchihewa 1994).

In one study of *A. mellifera* conducted in the Philippines, researchers found that the endemic bees, *A. cerana* and *A. dorsata*, negatively affected the growth of *A. mellifera* colonies in a forest ecosystem by aggression and robbing of stores. However, this finding was not duplicated for colonies studied in industrial or agricultural areas. Rather, the population growth of *A. mellifera* in an agro-ecosystem was significantly higher than in the industrial or forest environments. The abundance of melliferous plants in the agro-ecosystem enhanced the population build up of *A. mellifera*. They conclude that in spite of the diversity in a forest ecosystem, the exotic species *A. mellifera* failed to exploit the nectar and pollen sources of most plant species. This indicates that *A. mellifera* did not adapt to natural forest conditions in the tropics (Manila-fajardo and Cervancia 2003). In nestmate recognition experiments, *A. cerana* was among the bee species that did not exhibit aggressive responses to the presence of other bees in their nests (Breed Deng et al. 2007). These authors suggest that robbing of stored food may be more characteristic of *A. mellifera* than other species in the genus *Apis*. Similar reports appear in Ruttner's (1988) account of this species. In relation to *A. mellifera* robbing efforts, he writes that 'no effective defensive reactions are developed in *A. cerana*' (Ruttner 1988, p. 137). Indeed, the intruders can pass unimpeded, and *A. cerana* bees inside a robbed colony were observed feeding the intruding *A. mellifera* robber bees.

3.10.2 Colony Size and Abundance

There is divergent evidence about the size of *A. cerana* colonies. A recent report of a full-sized colony described it as containing about 1,500 g bees, 700 g brood, about 4 kg of honey and about 400 g of cells filled with pollen (Koeniger et al. 2010). Other reports cite usual colony sizes of 1,400–2,000 bees or between 10,000 and 20,000 bees (Makhdzir and Osman 1980; Ruttner 1988; Okada 1985 cited in Ruttner 1988). Colony size seems to depend on nest cavity availability. *A. cerana* tolerate a wide range of temperatures from 5 to 45 °C: however, when compared with *A. mellifera* at 50 °C, *A. cerana* survived for a much shorter time, whereas at 5 °C, they equaled *A. mellifera* survival rates (Verma and Edwards 1971 cited in Ruttner 1988). There is evidence from Ussuria, Kashmir, Japan and China that *A. cerana* are active at lower temperatures compared with *A. mellifera* and that they are therefore more active earlier in the morning than *A. mellifera* and can start flying earlier in spring than *A. mellifera* (Ruttner 1988). However, it should be noted that these data could be specific adaptations of certain ecotypes and may not be generalisable across whole species.

3.11 Reproductive Isolation Among Species of the Genus *Apis*

In the 1960s, research on reproductive isolation in honeybees started with the pioneering work of F. Ruttner on *A. cerana* and *A. mellifera*. Since then, the number of recognised *Apis* species increased from four to nine, and data on reproductive isolation played a key role in this development. The different mating behaviours include *behavioural mating barriers* (mating season, mating place, sexual signals, daily mating periods), *copulatory barriers* (size, genitalia, mating sign), *physiological barriers* (sperm transfer, sperm storage) and *postzygotic barriers* (fertilisation, development, hybrids). Allopatric *A. mellifera* and allopatric populations of the other species had a uniform mating period during the afternoon hours. Sympatric honeybee species were separated mainly by different daily mating periods. The mating period differed between populations of the same species from different regions. The sequence of the mating periods, however, described from Sri Lanka, Thailand and Sabah (Borneo) followed the same pattern and showed a taxonomic and size correlation: the dwarf bees (*A. andreniformis* and/or *A. florea*) occupied the first position shortly after noon. The next mating period was occupied by cavity-dwelling bees, and at sunset, *A. dorsata* drones flew out for mating. In addition, in the honeybee species that have been studied, various non-behavioural mating barriers have been demonstrated.

3.12 Dance Communication

The dance communication enables all species of *Apis* to monitor, through a small group of scout bees, the nectar and pollen sources in the surroundings. The information gathered by the scouts is 'evaluated' centrally in the colony, and the major force of foragers are directed to the optimal resources available at any given time. This 'information centre strategy' seems to be essential to understand the evolutionary success of honeybees (Seeley 1985).

A successful scout bee of *A. cerana* returns to the hive and, on the vertical comb, performs a dance which is similar to the dance of *A. mellifera* (Lindauer 1956). Food sources near the colony are indicated by round dances, typically performed by foragers running in circles and changing their direction from time to time. No information on the location of the food source is transmitted by the round dance. However, because the bees which follow the dance are in close contact with the dancer, they perceive the odour of the food source. This is, as demonstrated for *A. mellifera* (Frisch 1967), an important clue for finding the indicated food. Food sources at greater distances in Sri Lanka beyond 10 m are communicated by means of the waggle dance which contains information on the location of the food source. The direction to the food source is indicated by the direction of the waggle run. The direction to the sun serves as reference in the flight orientation. The vertical direction on the comb represents the direction to the sun and serves as a reference during the dance. This transposition of horizontal direction (flight angle) into the vertical face of the comb (dance angle) is common to *A. cerana*, *A. mellifera* and *A. dorsata*.

The distance of the food source correlates with the tempo of the dance. With increasing distances, the dance tempo becomes slower. In this regard, a comparison of *A. cerana* from Sri Lanka and *A. mellifera* from Europe (*A. mellifera camica*) was undertaken by Lindauer (1956). He found that the dance tempo of *A. cerana* was much faster than that of *A. mellifera* for the same distance. A more recent study by Punchihewa et al. (1985) confirmed this. A faster dance facilitates a better and more exact communication of shorter distance. The average foraging distance of *A. cerana* during those experiments in Sri Lanka was less than 200 m from the hive. Preliminary observations, made in Germany, of foraging distances of *A. cerana* from North Pakistan and China indicated larger foraging distances. Punchihewa was not able to train Sri Lankan *A. cerana* to feed beyond 500 m, and in the German experiments, foragers of *A. cerana* were easily trained to feed up to distances of 1,500 m. It is suggested that these differences within *A. cerana* represent adaptation to specific environmental foraging requirements. Thus, the faster waggle dance and the shorter flight range of Sri Lankan *A. cerana* is an adaptation to a specific habitat rather than a species-specific character. It is known that southern races of *A. mellifera* (*A. mellifera lamarckii* and *A. mellifera scutellata*) have a faster dance tempo than northern races such as *A. mellifera camica* (Frisch 1967).

3.13 Colony Defence

Differences in colony defence by hive bees vary from north to south. Southern, or more tropical, bees are more defensive than the northern races which are generally more gentle, e.g., the differences between African *A. mellifera scutellata* and the European *A. mellifera camica* are similar to the differences between *A. cerana* from Sri Lanka and northern Pakistan. The sting apparatus is the main defensive weapon. Its anatomy is very similar in both *A. mellifera* and *A. cerana*, but there are some differences. The barbs of the lancets are less developed in *A. cerana* than in *A. mellifera* (Weiss 1978). Further, the mechanism of sting autotomy is less expressed in *A. cerana* (Sakagami 1960b); thus, in these bees, the sting more often remains connected after use. The quantity of the main alarm pheromone, isopentyl acetate, is less in *A. cerana*. For *A. mellifera*, 2.3 μg of isopentyl acetate per sting was found, but it was only 1.5 μg for *A. cerana* (Koeniger et al. 1979). The main polypeptide from the venom is melittin and there is no difference in its amino acid sequence between *A. cerana* and *A. mellifera* (Kreil 1973).

The defence behaviour of *A. cerana* has some elaborate elements which are apparently missing in *A. mellifera*. A distinct reaction of *A. cerana* to optical stimuli, like flying insects, is described by several authors (Butler 1954; Sakagami 1960a; Schneider and Klotz 1971; Koeniger and Fuchs 1975). The bees react by a fast and sudden lateral body shaking. This behaviour can be observed readily when groups of guard bees react to hornets or wasps hovering at the nest entrance. Further, body shaking is frequently observed on swarms of *A. cerana*. The bees on the surface of the cluster react with simultaneous shaking behaviour and prevent the landing of flying insects on the cluster. Experiments showed that the speed (0.5–5 m/s) corresponds well to flight speed of the intruding insects. The size of the optical stimulus is important for eliciting this reaction, with larger stimuli causing greater response. Further, *A. cerana* has been shown to react continuously to an optical stimulus for more than 90 min (Koeniger and Fuchs 1975). This behaviour seems to be a well-adapted defence against flying insects. Preventing intruders from landing at the hive entrance or on the swarm cluster is more 'economic' and less risky than stinging, which, in the case of defence against wasps, always includes some risk of death.

Another defence behaviour of *A. cerana* can be elicited by mechanical stimuli. On a slight knock to the hive, the colony answers with a conspicuous hissing sound. This communicative sound production is transmitted from one bee to the other by body contact or by air movement produced by the wings. The speed of the transmission is fast (25 cm/s) (Fuchs and Koeniger 1974). The colony or a swarm cluster reacts as a unit and the sound is highly audible. Preliminary experiments with an Asiatic bear in a zoological park near Oberursel demonstrated the effect of the behaviour. The bear was trained to find a honey comb in a small wooden box. Then, in the experiment, the hissing sound of a colony of *A. cerana* was emitted from a loudspeaker in the box by the first touch of the bear. The animal was repelled at once. This suggests that hissing out of a dark box or cavity can be understood by larger vertebrates as a danger (snake's) signal! This kind of mimicry may be supported by the rapid decrease of

the bees' activities and flights after the hissing reaction. A further, very spectacular, defence behaviour of *A. cerana* was recently reported from Japan (Ono et al. 1987). Upon the approach of flying hornets, guard bees formed groups and stayed in tight contact with each other. When the hornet attacked, the bees stayed together and formed a 'ball' around the hornet. After several minutes, they released the hornet.

Much has been written about colony defence behaviours and the consensus for *A. cerana* is that it is generally reported as being mild, tolerant and timid (Ruttner 1988) in the context of attacks from European genotypes of *A. mellifera*. However, there are some unique behaviours associated with this species associated with colony defence: (a) abdomen shaking, (b) hissing (like a snake) in response to knocks or interference with the combs, (c) group defence via mob capture of large wasps near the nest entrance and (d) stinging behaviour. In Japan, Ono et al. (1995, cited in Oldroyd and Wongsiri 2006, p. 201) noted one additional colony defence behaviour in response to attack from a hornet: recognition and removal of a marauding wasp pheromone before it has a chance to attack other hornets. There is an important early paper by Sakagami which outlines the competition and interaction of *mellifera* and *cerana* honeybee species in observations at mixed colonies (Sakagami 1959). In Japan, the endemic *cerana* species was gradually replaced by *mellifera* with records of *cerana* extinction dating back to 1925, since *mellifera* were first introduced into that country in 1876. Japanese (alongside many other) apiarists preferred the introduced *mellifera* species given the ease with which they adapted to movable frames and their greater honey production.

Sakagami summarised that, in general, *A. cerana* is more tolerant and less aggressive than *A. mellifera*. He writes that with respect to interspecific conflict (in mixed colonies) *A. mellifera* usually took the dominant position in both aggressiveness and agility. He notes the superiority of (*mellifera*) species in terms of their larger colony size, strong fighting capacity and protection afforded by humans. Another difference between the two species was noted in terms of their foraging behaviour. Citing Hachinow 1954, Sakagami writes: (*mellifera*) have a tendency to concentrates their effort on a major nectar source whilst (*cerana*) tend to forage from numerous minor sources. He also makes the point that under natural conditions, the species would interbreed very rarely, if at all. Aligning with this assessment of less aggression of *A. cerana* compared with *A. mellifera* is the evidence of their stinging behaviour. Oldroyd and Wongsiri wrote that *A. cerana* are more likely to retreat inside the nest than to sting on the approach of a mammal (2006). In one experiment, however, *A. cerana* did sometimes attack an intruder (an artificial mouse made of felt)—afterwards no stings were detected in the felt—whereas *A. mellifera* stings were extensive on the same target. Moreover, the sting of an *A. cerana* worker bee contains about half the quantity of stinging material (isopentyl acetate) compared with those of *A. mellifera* worker bee stings. However it has been reported that *A. cerana* stings have a considerably longer effect than *A. mellifera* stings. Finally, *A. cerana* appears to have the least well-developed barbs on the sting lancet compared with all other *Apis* species barbs (Ruttner 1988).

3.14 Mating Behaviour

3.14.1 Drone Development

The drone cells, which are larger in diameter (7 mm in Japan) than worker cells (4.8 mm in Japan) (Okada and Sakai 1960), are found mostly at the lower portion of the comb. The queen starts laying eggs into drone cells at the beginning of the flowering season. Towards the end of the drones' larval development, workers seal the cell with a wax cover. The larva spins its cocoon before metamorphosis starts. After some days, the bees remove the wax from the cell capping and a dome-shaped cover with its central pore is exposed. Apparently, the pore, which is found only in *A. cerana*, is the result of a locally applied secretion by the drone larva (Haenel and Ruttner 1985). Its function is not known. The total development of the drones of *A. cerana* takes 21–24 days in India (Singh 1962) which is longer than worker development at about 18–20 days (Singh 1962). The cappings of the drone cells are opened by the emerging drones and fall to the hive floor where they usually remain. The time of drone flight varies among locations. In Pakistan (North-West Frontier Province), drones flew between 1,100 and 1,500 h and in Sri Lanka (North-Central Province) the drone flight was restricted to 1,615–1,715 h (Koeniger and Wijayagunasekera 1976). The number of sperms produced by drones of *A. cerana* is significantly smaller than that in *A. mellifera*. The vesiculae of *A. cerana* contained 1.5 million sperm but those of *A. mellifera* contained about 11 million sperm (Ruttner et al. 1973).

3.14.2 Queen Development

Queen cells in *A. cerana* are constructed at the edge of the comb. They have the typical form and vertical orientation as in all *Apis* species. The development of the queen takes 15–16 days in India (Singh 1962). The number of queen cells per colony varies according to its strength. In Pakistan and Sri Lanka, it was found that some colonies, after the first swarm had left, had more than 20 sealed queen cells, while others in the same location had only two to five. Mating time in India (Pune) is between 1,400 and 1,500 h and the mean duration of the 'successful' flight was 27 min (Woyke 1975). The success of the mating was easily determined by the percent mating sign which protruded from the queen's sting chamber. It consists, in *A. cerana*, of a mucus plug and an orange sticky layer. Woyke (1975) dissected young queens returning from their first mating flight. The average volume of semen found in the oviduct of the queen was 1.94 mL. Because *A. cerana* drones produce about 0.2 mL of semen, it can be concluded that there were about 10 copulations during the mating flight. On average, the spermatheca of naturally mated queens of *A. cerana* contained 1.3 million spermatozoa, that is only one-quarter the number found in *A. mellifera carnica*. Different results were reported from experiments in

Germany with *A. cerana* from Pakistan. There, about 3.5 million sperm were counted in spermatheca of a queen *A. cerana* (Ruttner and Maul 1983).

Although drones of *A. cerana* seem to produce only 10 % of the amount of sperm found in drones of *A. mellifera*, there is only a small difference in the number of matings per mating flight: ten in *A. cerana* and eight in *A. mellifera carnica*. The amount of semen found in the spermatheca of *A. cerana* is about 25 % of that in *A. mellifera*. Thus, the mechanism of the sperm transfer from the oviducts into the spermatheca must be twice as efficient in *A. cerana*. Of about 10 million sperm which are received in the oviduct of *A. cerana*, about 13 % reach the spermatheca, whereas in *A. mellifera*, 80 million are in the oviduct and only 7–8 % are found in the spermatheca.

3.14.3 Swarming

In *A. cerana*, reproductive swarming is similar to *A. mellifera*, for example, *A. cerana* reproductive swarms settle at 20–30 m away from the natal nest (natal nest means mother or primary colony), stay for a few days and then depart for a new nest site, after getting information from the nest-locating scout bees. Scout bees begin searching for suitable cavities in which the swarm's home can be constructed. Successful scouts come back and report the location of suitable nesting site to other bees by performing communication dances on the surface of the swarm cluster in the same way they would do for food sources.

In many areas of subtropical and tropical Asia, the flowering season depends on monsoon rains which usually occur twice a year. In these areas, *A. cerana* has two swarming seasons. In Sri Lanka (North-Central Province), for example, the first swarming takes place in March and April. This is after the rains which usually come in the middle of December. The second swarming season is in July and August after the summer rains. Normally, swarming starts at the end of or somewhat after the peak of the flowering season. The colonies have then collected enough honey to be 'invested' in colony multiplication by swarming. In Japan and temperate China, *A. cerana* has only one swarming season per year. The number of swarms per colony seems to be higher in tropical and subtropical races. In Sri Lanka and Pakistan (Latif et al. 1960) three to eight swarms are reported, whereas Tokuda (1971) found only one to three swarms in Japanese *A. cerana*. Swarms of *A. cerana* vary greatly in size. The prime swarms consist of up to 10,000 bees, whereas some after-swarms are quite small: several times we counted less than 2,000 bees in North-West Frontier Province, Pakistan! A high percentage of swarms which were captured and put into hives absconded, sometimes even after they stayed for several days and initiated comb-building.

3.15 Absconding and Dispersal (Migration)

Absconding is the deserting of the nest and the combs. In many tropical bees, it is a common reaction to a decreasing environmental 'quality'. Scarcity of nectar and pollen sources (often caused by drought) diminishes or terminates brood rearing. Under these conditions, honeybee colonies of all four species react to any kind of disturbance (wax moth infestation, honey harvesting by man, predation by hornets, etc.) by absconding.

In tropical *A. cerana*, absconding occurs at the end of the swarming season. By then, most of the bees have left the nest with previous swarms. The combs of the nest are no longer covered by bees and are immediately attacked by wax moths. This results in absconding. However, colonies of *A. cerana* which did not swarm previously also abscond easily. Often, viable eggs and young larvae are left behind by *A. cerana* of southern India (Woyke 1976). Migration, in the sense of a regular long distance movement from one location to another, far away, is a well-documented phenomenon in the giant honeybee, *A. dorsata*. The colonies leave from and return to habitats seasonally as migratory swarms (Koeniger and Koeniger 1980).

In the other species, particularly in the tropical races, the colonies abscond and leave locations and areas whenever conditions become 'intolerable'. However, the direction of the moving swarms and the orientation does not seem to be consistent. The colonies of *A. cerana* seem to disperse rather than migrate. This seems to be the case in Sri Lanka (Anuradhapura) and in Pakistan (Peshawar). Also, a report of Woyke on *A. cerana* from India (Pune) indicated that there was no directionality detectable in moving swarms of *A. cerana* there. Field studies in areas with distinct sequences in flowering pattern, such as steep valleys, are recommended to decide whether a regular migration may exist in *A. cerana* under special environmental circumstances.

3.16 Nest Thermoregulation

A. cerana maintains internal hive temperatures with a precision similar to that of *A. mellifera*, using similar mechanisms. *A. cerana* colonies maintain their blood temperature in the range of 33–35.5 °C even when ambient temperatures vary between 12 and 36 °C. This mechanism clearly shows that they possess effective nest thermoregulation systems. During summer, *A. cerana* employs evaporative cooling, where the worker bees cluster outside the nest in hot weather and fan their wings, thus removing excess heat and moisture from the nest and decreasing the hive temperature. Thermal defence: when an *A. cerana* hive is invaded by the Japanese giant hornet (*Vespa mandarinia*), about 500 Japanese honeybees (*A. cerana japonica*) surround the hornet and vibrate their flight muscles until the temperature is raised to 47 °C (117 °F), heating the hornet to death, but keeping the temperature still under their own lethal limit (48–50 °C). European honeybees (*A. mellifera*) lack this behaviour.

Kraus et al. (1998) compared temperature profiles in the brood nests of *A. mellifera mellifera* and *A. cerana indica* and found similar temperatures within the centre in worker brood cells, and external temperatures between 18 and 33 °C. At the periphery of the brood nest, where drone brood usually is located, temperature in brood cells of *A. cerana* was clearly lower compared with *A. mellifera*. Average temperature in drone brood cells was 0.4 °C lower compared with nearby worker brood cells. While temperature in worker brood cells of *A. mellifera* colonies was close to 35 °C, temperature in drone brood cells of *A. cerana* colonies was only 33 °C, at ambient temperatures in Sabah (Malaysian Borneo).

3.17 Coexistence and Interaction With the Other Asian Honeybee Species

Competition among closely related species can be disastrous, so the question of how all three species *A. cerana*, *A. dorsata*, and *A. florea* manage to lie closely together has sparked some research. Competition for nesting sites does not occur because *A. cerana* is the only species which needs cavities for the construction of its nest. Also, interaction among the honeybees during mating seems to be avoided. In Sri Lanka, a time-sharing mechanism prevents an interspecific overlap of mating. Thus, it is assured that the queen's pheromone, 9-oxo-2-decenoic acid, will always be directed at the drones of the same species.

Some experiments on food competition were made in Sri Lanka (Koeniger and Vorwohl 1979). Bees of four species, a meliponid bee *Trigona iridipennis*, *A. florea*, *A. dorsata* and *A. cerana* were trained to feed at a common feeding dish simultaneously. *A. cerana* was more competitive than *A. dorsata* but less so than *A. florea*. Furthermore, the intraspecific fights among foragers of different colonies of *A. cerana* were significantly more frequent and of higher intensity than those of the other bees. A comparative analysis of pollen from honey samples of each species allowed conclusions on natural foraging habits to be drawn. The smaller bees (*Trigona* and *A. florea*) seemed to forage on many plant species within their small flight range. They showed a higher tendency to defend the feeding dish. *A. dorsata* did not show much defensiveness at the feeding dish. Honey of *A. dorsata* contained pollen of only a few plant species. This implies that it seems to concentrate its foraging activities on the more profitable flowers within its larger flight range. Honey of *A. cerana* contained a similar diversity of pollen grains as did that of *A. florea*. Thus, competition between the species for floral resources is reduced by different foraging strategies. Moreover, the annual migratory cycle of *A. dorsata* contributes to further reduce the competition once these bees have left the area entirely during dearth. Although these studies are only in the initial stages, one gets the impression that the relationship among the Asian honeybee species is well balanced and that competition is avoided (mating) or at least reduced (food).

3.18 Manmade Coexistence Between *A. cerana* and *A. mellifera*

With the successful establishment of *A. mellifera* in an Asian region, the indigenous *A. cerana* disappears. Good examples of this phenomenon are from Japan, some parts of India (Punjab) and some areas of Pakistan. The start of this competition between both species is 'manmade' because of import of the bees to areas outside their natural range. In addition, in the course of the establishment of *A. mellifera*, beekeepers, with their chemicals and other ways of protecting their honey producers, play an active and essential role. Therefore, *A. mellifera* is not the biologically 'dominant' species in Asia and it will remain there only in total dependence on humans. Even after beekeepers have destroyed many of its competitors, predators and other natural 'problems', it will not survive without constant care.

The main problems of coexistence result from the similarity and general biological 'overlap' of both species. The best documented case is interspecific competition during mating. The species which has smaller numbers of drones at the congregation area fails in mating. Imported *A. mellifera* did not produce mated queens (Akkratanakul 1976; Ahmad 1984) in Asia and imported *A. cerana* did not successfully mate in Germany. These problems can be solved by isolation of the imported species from indigenous bee colonies.

A further problem results from interspecific robbing behaviour. Although 'strong' colonies of hive bees normally defend themselves well against conspecific robbers, they have some inappropriate reactions in the case of heterospecific robbers. We observed that defending guard bees of *A. cerana* actually fed robbing foragers of *A. mellifera*. The latter then, because of the foraging success, recruited large numbers of additional robbers. At the end, the hives of *A. cerana* were regularly invaded and destroyed. In Pakistan, robbers of *A. cerana* were seen entering colonies of *A. mellifera*; however, losses of colonies of the latter as a result of robbing by *A. cerana* occurred rarely. Robbing colonies of *A. mellifera* by *A. dorsata* was much more disastrous.

Great problems occur from interspecific exchanges of parasites. The natural adaptation protects only the original host species. For the new species, the heterospecific parasite can cause severe damage. Examples of *A. cerana* suffering from parasites of *A. mellifera* are Thai Sacbrood Virus (Verma 1985) and *Acarapis woodi* (Ahmad 1984), and the reverse cases are *A. mellifera* and the damage caused by the parasitic mites *Varroa jacobsoni* of *A. cerana* and *Tropilaelaps clareae* of *A. dorsata*.

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Chapter 4

Biogeography

4.1 Geographic Distribution

Apis cerana, or the Asiatic honeybees are small honeybees of southern and south-eastern Asia, such as China, India, Japan, Malaysia, Nepal, Bangladesh and Papua New Guinea. This species is the sister species of *Apis koschevnikovi*, and both are in the same subgenus as the European honeybee, *Apis mellifera*. For ages, colonies of the oriental honeybee *A. cerana* have provided mankind with honey and beeswax, as well as furnishing invaluable service in the pollination of agricultural crops. This bee's range of distribution is far greater than those of *Apis florea* and *Apis dorsata*, it is found throughout the tropical, subtropical and temperate zones of Asia, occurring in the Indian subcontinent and Sri Lanka in the west, through Southeast Asia, to Indonesia and the Philippines in the east. Further north, it is found in the southern USSR and China, through the Korean peninsula, to Japan. This wide range has led to important variations among the bee's geographical races, particularly between the tropical and temperate races, there are wide differences in workers' body size, nest size, colony population, swarming and absconding behaviour. The temperate and subtropical races appear to store greater quantities of food than the tropical races, which in turn are more mobile than the former, tending to swarm, abscond and migrate quite frequently.

A. cerana, the indigenous hive bee of Asia, is the most valuable natural resource of beekeeping and has been considered a vital component of the natural ecosystem. It is well adapted to the local climatic conditions and floral resources through centuries of natural selection. It has been reported to be an excellent pollinator of crops that bloom in early spring, such as almonds, apples, pears, plums and different vegetable seed crops. Beekeepers and pollination scientists have been experiencing a rapid decline in *A. cerana* populations which may result in the loss of plant biodiversity in an area and create socio-economic problems. Because of the decline of *A. cerana* colonies in many regions of Asia, *A. cerana* is an endangered species. The smaller the native population of *A. cerana* in any area, the higher the danger for this bee because of its mating behaviour. When the *A. cerana* population is destroyed, a native and well-adapted pollinator for both native and agricultural plants will be lost. The results

for loss of native plant biodiversity and the pollination of agricultural crops cannot be estimated because *A. mellifera* is not able to pollinate as effectively as *A. cerana*.

The geographic distribution of *Apis* species in the Oriental region was extensively reviewed by Maa (1953). According to him, the primary distribution centre was in the Malayan subregion, where not only the number of species was greater than elsewhere, but also the various degrees of specialization co-existed. He further suggested that the subgenus *Apis* included honeybees from the Oriental, Palearctic and Manchurian subregions. In all, he described more than ten species. Michener (1974) reported a very wide distribution of *A. cerana* in Southeast Asia, extending from Ceylon and India to Japan and southeast to Moluccas. Kellogg (1938) discussed the distribution of *A. cerana* in China. He considered the bees in China to be a variety of *Apis indica* and different from the Japanese variety. *Apis indica pernoi* was the largest variety of Oriental honeybee found in Fukan Province of southeast China. According to Sakagami (1959), the native *A. cerana* in Japan was being replaced by the exotic European honeybee *A. mellifera*, but still the former species was found in the mountainous regions both in the wild state and also in hives. Sakai and Matsuka (1982) reported wide occurrence of *A. cerana* throughout Japan except in the northernmost island of Hokkaido. Distribution of *A. cerana* in various parts of Afghanistan, Philippines and Sri Lanka have been given by Schneider and Djalal (1970), Morse and Laigo (1969) and Fernando (1979) respectively.

The distribution of honeybees in different parts of India has been studied by various investigators from time to time. Hussain (1939a, b) claimed that honeybees originated in India. He described three species of honeybees from India, rock bee (*A. dorsata*), indigenous bee (*A. indica*) and pigmy bee (*A. florea*). Rahman and Singh (1940) reported *A. cerana indica* from all over India. These authors distinguished the smaller and yellowish plain strains and larger and darker hill strains of this species. Rahman (1944) for the first time showed the presence of *A. cerana indica* at a height of 3,030–3,333 m in a wild state. Muttou (1944, 1956) claimed India to be the place of origin of the genus *Apis*. He described the following species of honeybees in India: *A. indica*, *A. dorsata*, *A. florea*. The hill variety of *A. indica* was named as *Apis indica gandhiana*. Sharma (1945) described the distribution of *A. indica* in the plains of northern India and reported the absence of this species from the Rajputana desert. Lal (1945) described three different species of honeybees in India, and two strains of *A. florea* (one smaller and another bigger strain) were reported from Bhopal. Deodikar (1959) while studying the ecotypes of *A. cerana* found a gradual variation in some characters such as body size, tongue length and number of hamuli. The value of these characteristics was sometimes akin to the European bee, *A. mellifera*. He also showed differences in the genitalia of *A. cerana* from the northern and southern regions of India. Narayanan et al. (1960a, b, 1961) suggested the existence of three races of *A. cerana* in India which were distinguished as Himalayan, the Gangetic plains and the South Indian plains races. Singh (1964) reported *A. cerana*, *A. dorsata* and *A. florea* at elevations of 2,424, 1,515–2,424 and 606–757 m, respectively.

Ruttner (1985, 1986, 1987) recently reviewed the geographic distribution of *A. cerana*. According to him, this species is found in a very wide area comprising mainly southern and eastern Asia. In the west, it extends from Afghanistan up to the Philippines in the east and in the north from Ussuria to Java in the south (Fig. 4.1). Thus, *A. cerana* is found not only in the tropical and subtropical regions of Asia,

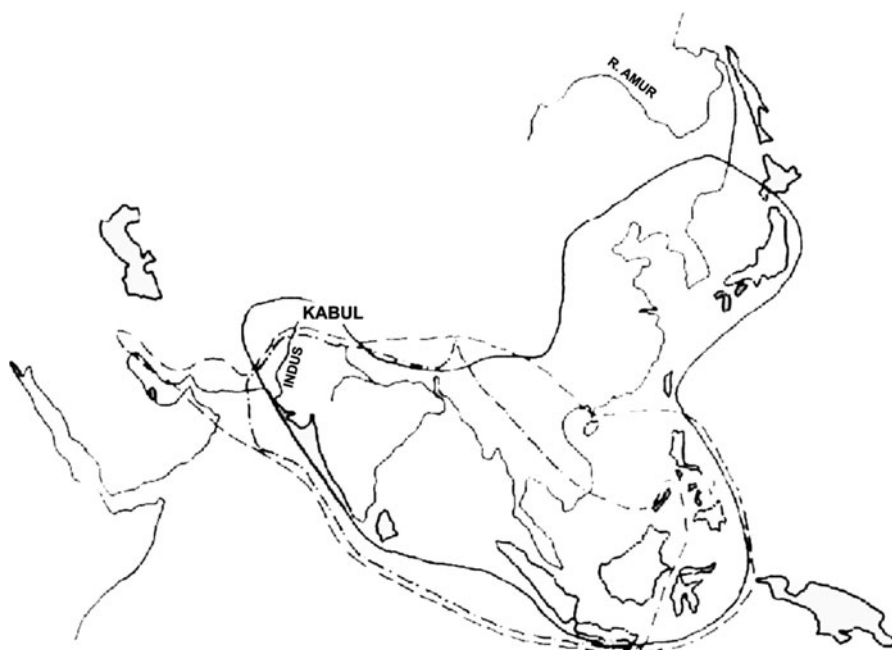


Fig. 4.1 Geographic distribution of *Apis cerana* (——) *Apis florea* (-----) and *Apis dorsata* (-·-·-·-)

but also in cooler climate such as Siberia, northern China and higher altitudes of the central Asian Mountains (Koeniger 1976b).

Through the centuries, as a result of the continuous process of natural selection, different subspecies and geographic ecotypes of honeybees have been evolved. Such intraspecies diversity in honeybees can be exploited for commercial purposes due to the phenomenon of heterosis. It is now well documented that by crossing different subspecies of *A. mellifera*, honey production can be increased by 50–116 % (Fresnaye and Lavie 1976). Similarly, substantial genetic differences existing at subspecies and ecotype level has been exploited through selective breeding techniques to develop high and low preference lines for pollination of agricultural crops, resistance to diseases, increased pollen gathering and enhanced longevity (Kulincevic 1986). A brief account of different subspecies and geographic ecotypes of *A. cerana* identified so far in different regions of its native habitat is given below.

4.2 Subspecies and Geographic Ecotypes

4.2.1 Western Asia

Till the year 1985, very little was known about the different subspecies and geographic ecotypes of *A. cerana* in its native habitat. The earlier biometric investigations especially in India were based on a few morphological characters and geographic

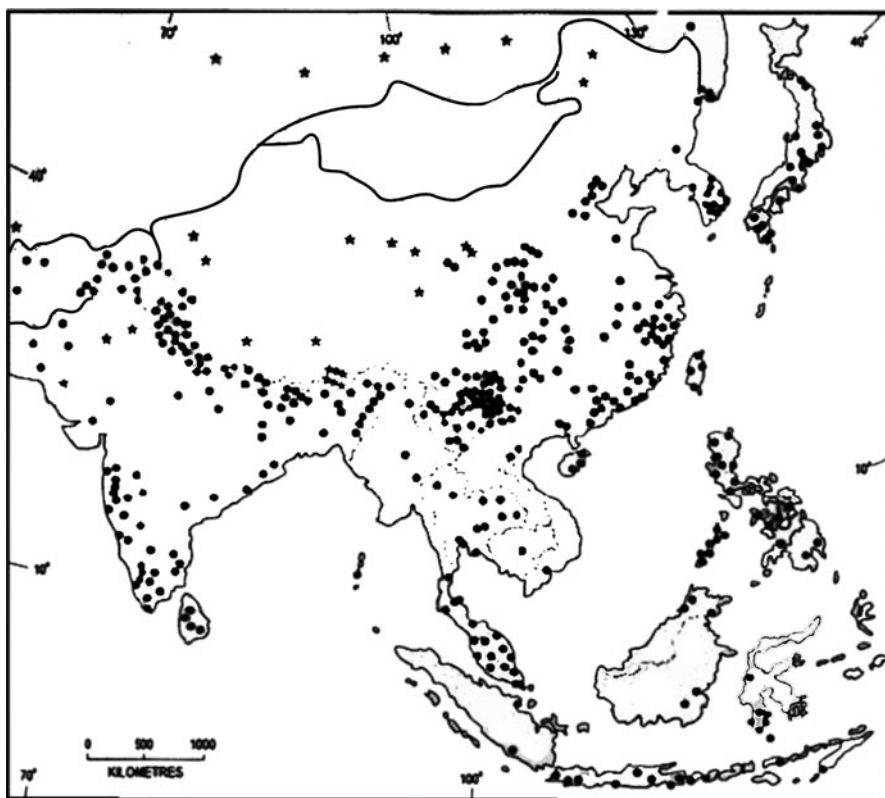


Fig. 4.2 Known distribution of *Apis cerana* (circles); reported absence (stars)

samples, and also lacked proper statistical analysis of data (Kapil 1956; Narayanan et al. 1960a, b, 1961; Kshirsagar 1976; Venkatasubbya 1938; Ratnam 1939; Rahman and Singh 1948; Jaggannadham and Goyal 1980; Kshirsagar and Ranade 1981; Morinioto 1968; Kshirsagar 1973; Maa 1953; Sakai 1958; Akahira and Sakagami 1959b; Jain 1967; Okada et al. 1956; Bingham 1897).

The first accurate morphometric analysis on *A. cerana* to identify different subspecies and geographic ecotypes by using morphological characters and computer assisted standard statistical methods was made by Ruttner (1985, 1986, 1987). He has distinguished four different subspecies of *A. cerana* (Fig. 4.2) and these are:

1. *Apis cerana cerana* distributed over north China, north-west India, northern Pakistan and Afghanistan.
2. *Apis cerana himalaya* from north-east Himalaya
3. *Apis cerana indica* from South India, Sumatra, Malaysia, Sri Lanka, Java and Thailand.
4. *Apis cerana japonica* from Japan.

Ruttner (1985) reported that this subspecies can further be subdivided into two separate ecotypes or subgroups, i.e. Honshu (Tokyo region) and Tsushima bees. These two ecotypes differ from each other in tongue length, hair length and colour patterns. These subspecies also have a higher cubital index and slender abdomen as compared to subspecies of *A. cerana*.

Verma and his students published a series of research papers on multivariate analysis of morphometric traits of *A. cerana* measured from 3,704 individual worker bees of 279 colonies from 64 localities with an average sampling distance resolution of 50 km along a 2,200 km tract in the Himalayan region bordered by Pakistan to the west and Myanmar to the east. The localities from which *A. cerana* were collected along a 2,200 km transect in the southern Himalayan region are given in Table 4.1 and details of this morphometric analysis are published in a monograph (Verma 1995).

Verma and his students (Verma et al. 1989, 1994; Singh et al. 1990; Singh and Verma 1993) used 55 quantitative morphological characters drawn from the earlier work of Ruttner (1987) and Alpatov (1929) on *A. mellifera*. However, Hepburn et al. (2001a, b) re-examined the earlier list of Ruttner (1987) and Alpatov (1929) by performing a factor analysis of the colony means using these 55 characters for all 3,704 bees collected across the entire transect. This procedure established which characters have larger loadings in the various factors and allows the parsimonious reduction in the number of characters actually needed for further analysis. The next procedure was a correlation analysis of the colony means for all 55 characters to determine which characters were highly correlated. If two or more characters with high factor loadings (greater than 0.6) were highly correlated ($r > 0.8$), then only one was selected for further analysis. Finally, a factor analysis for the whole transect using colony means for the remaining 22 selected characters was performed, followed by a discriminant analysis of colony means for these 22 characters to determine colony groupings. A jackknife procedure was used to classify each colony into a morphocluster with the highest posterior probability according to the discrimination function (Lachenbruch and Mickey 1968). Wilks' Lambda statistic was used to test for significant differences between the vector of means of the characters entered into the discriminant functions. The inter-colonial variances at each locality were tested for heterogeneity by means of Levene's F Statistic (Johnson and Wichern 1998).

A factor analysis using the colony means of the selected 22 characters of worker honeybees from the entire transect isolated three factors with Eigen values greater than one. Factor 1: characters associated with the size of the honeybees; Factor 2: angle of venation (10); and Factor 3: angles of venation (11) and (13). These three factors accounted for 78.6 % of the variance in the data. The loadings for each character had an absolute value greater than 0.6. A graph of the factor scores revealed four morphoclusters: colonies from the Kashmir region formed one cluster (I), those from the Himachal region formed a second cluster (II), those from Uttar Pradesh and Nepal regions formed a third cluster (III), and those from the northeast Himalayan region formed a fourth cluster (IV) (Fig. 4.2). The colonies from two localities at high altitudes, Ghughuti (2,470 m) and Vinaula (2,895 m), formed their own cluster. The 5 colonies from Lucknow (30) appeared in the far left-hand of the third morphocluster indicating that the bees from this region are smaller and probably belong to a southern

Table 4.1 Localities from which *Apis cerana* were collected along a 2,200 km transect in the Southern Himalayan region

No.	Localities	Coordinates	Altitude	Swarming time
1	Kupwara	34.31° N 74.16° E	1,811	April
2	Gurais	34.37° N 74.53° E	2,364	April
3	Tral	33.56° N 75.10° E	2,007	April
4	Dras	34.26° N 75.46° E	2,977	April
5	Srinagar	34.08° N 74.50° E	1,768	April
6	Sonamarg	34.18° N 75.21° E	2,740	April
7	Rajouri	33.23° N 74.21° E	938	February
8	Kishtwar	33.19° N 75.48° E	1,664	April
9	Dalhousie	32.32° N 75.58° E	2,036	April
10	Kangra	32.05° N 76.16° E	700	February
11	Katra	32.05° N 77.08° E	1,463	March
12	Pooh	31.40° N 78.34° E	2,837	April
13	Bhareri	31.23° N 76.34° E	1,007	March
14	Mandi	31.43° N 76.50° E	761	February
15	Roghi	31.32° N 78.15° E	3,017	April
16	Bilaspur	31.15° N 76.40° E	587	February
17	Bagi	31.15° N 77.27° E	2,648	April
18	Shimla	31.07° N 77.10° E	2,206	April
19	Solan	30.50° N 77.08° E	1,530	April
20	Nahan	30.33° N 77.21° E	905	February
21	Dehradun ^a	30.30° N 78.08° E	762	February
22	Haridwar ^a	30.02° N 78.04° E	330	February
23	Pauri ^a	30.12° N 78.48° E	1,550	April
24	Chaubattia ^a	29.55° N 79.00° E	2,122	April
25	Almora ^a	29.36° N 79.40° E	1,750	April
26	Nainital ^a	29.23° N 79.32° E	1,920	April
27	Jeolikote ^a	29.16° N 79.46° E	1,250	March
28	Haldwani ^a	29.13° N 79.31° E	800	February
29	Budaun ^a	28.02° N 79.07° E	150	February
30	Lucknow ^a	26.50° N 80.54° E	150	February
31	Lohaghat ^a	29.25° N 80.06° E	1,200	March
32	Lali ^a	29.49° N 80.36° E	1,097	March
33	Sharmali ^a	29.14° N 80.25° E	1,635	April
34	Navadurga ^a	29.15° N 80.27° E	1,837	April
35	Lanakedareswar ^a	29.22° N 80.56° E	1,360	March
36	Durg ^a	29.10° N 81.20° E	1,345	March
37	Ghughuti ^a	29.18° N 82.12° E	2,470	April
38	Vinaula ^a	29.18° N 82.18° E	2,895	April
39	Liwang	28.25° N 82.40° E	1,500	April
40	Laltibang	28.20° N 82.30° E	900	February
41	Ghorahi	28.05° N 82.20° E	400	February
42	Lumle	28.20° N 84.00° E	1,400	March
43	Tansen	27.55° N 83.40° E	1,067	March
44	Sidheshwar ^a	27.18° N 85.56° E	1,463	March
45	Suspa ^a	27.44° N 86.10° E	1,600	April
46	Letang	26.40° N 87.25° E	250	February
47	Homtang	27.10° N 87.10° E	600	February
48	Virgaon	27.05° N 87.51° E	1,200	March
49	Kurseong	26.56° N 88.20° E	1,458	March
50	Gangtok	27.02° N 88.40° E	1,818	April

Table 4.1 (continued)

No.	Localities	Coordinates	Altitude	Swarming time
51	Bongaigaon	26.28° N 90.32° E	76	February
52	Khoirabari	26.38° N 91.51° E	134	February
53	Guwahati	26.11° N 91.47° E	56	February
54	Shillong	25.34° N 91.56° E	1,496	March
55	Silchar	24.50° N 92.51° E	67	February
56	Aizawl	23.45° N 92.45° E	1,132	March
57	Itanagar	27.08° N 93.40° E	550	February
58	Dimapur	25.54° N 93.48° E	160	February
59	Kohima	25.41° N 94.06° E	1,495	March
60	Mao	25.30° N 94.07° E	2,012	April
61	Ukhrul	25.08° N 94.23° E	1,829	April
62	Imphal	24.49° N 93.58° E	792	February
63	Moirang	24.30° N 93.48° E	782	February
64	Churachandpur	24.20° N 93.40° E	914	February

^aNew localities

Indian morphocluster not defined here. The graph of the factor scores confirms the results of Verma et al. (1989, 1994) that Nepali bees are smaller in size than Kashmiri and Himachali bees, but bigger than Manipuri bees.

Factor and discriminant analysis revealed four major (morphoclusters) unnamed subspecies. Therefore, morphoclusters/biometric groups/subspecies occur as follows:

- I. Kashmir
- II. Himachal Pradesh
- III. Uttar Pradesh and Nepal
- IV. Sikkim, Bengal and NE states of India

Morphoclusters III and IV are further resolved into three biometric subgroups each from west to east as follows:

- III a. Uttar Pradesh
- III b. West Nepal
- III c. Central and east Nepal
- IV a. Sikkim, Bengal, Assam and Arunachal Pradesh
- IV b. Assam and Meghalaya
- IV c. Nagaland, Manipur and Mizoram

Names of localities are given in Table 4.1.

Thus the composite structure for *A. cerana* in the Himalayan region reveals four primary, major morphoclusters (subspecies?), two of which can be further resolved into three biometric subgroups. These results are the products of statistical analysis of morphological variation for the region. On reflecting on the clusters shown in Fig. 4.3, Verma (1995) suggested that the morphological groups might well be biologically meaningful because they are geographically separated. However, in biological terms the four morphoclusters and six biometric subgroups of Fig. 4.3 still require explanations as to their biological meaningfulness as separate populations.

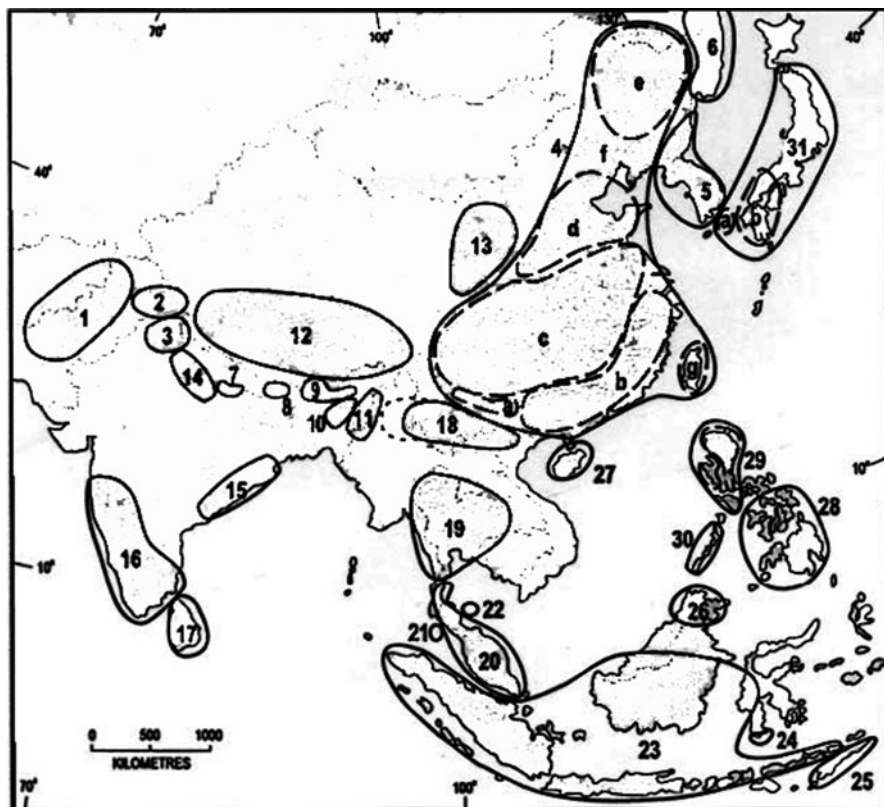


Fig. 4.3 Distributional areas of putatively distinct subspecies, biometric groups and/or ecotypes of *Apis cerana*. *Apis cerana cerana*: 1 eastern Afghanistan and northern Pakistan (by inference only), 2 Kashmir, 3 Himachal Pradesh, 4 China (with biotypes/ecotypes *a* Yunnan, *b* Guangdong-Guangxi, *c* Hunan, *d* northern, *e* Changbei Shan, *f* unspecified, *g* Taiwan), 5 Korea, 6 Ussuria. *Apis cerana himalayana*: 7 Nepal Terai plains, 8 Nepal midlands, 9 Himalayas, 10 Brahmaputra, 11 Manipur, Mizoram and Nagaland. *Apis cerana skorikovi*: (possibly *Apis cerana cerana*) 12 Tibet. *Apis cerana abaensis*: 13 Central China. *Apis cerana indica*: 14 Uttar Pradesh, 15 Orissa, 16 Southern India, 17 Sri Lanka (with montane, lowland and Anuradhadpura ecotypes), 18 Yunnan and possibly northern Myanmar, 19 Northern Thailand, 20 Southern Thailand and continental Malaysia, 21 Phuket Island, 22 Samui Island, 23 Sumatra (northern half by inference only), Java, Borneo, Lombok, Bali, Flores and most of Sulawesi, 24 Southern Sulawesi, 25 Timor, 26 Sabah. *Apis cerana hainanensis*: 27 Hainan Island (with coastal and montane ecotypes). *Apis cerana philippina*: 28 Visayas and Mindanao, 29 Luzon (with highland and lowland ecotypes), 30 Palawan (distinguishable from other Philippine morphoclusters). *Apis cerana japonica*: 31 Japan (with two ecotypes). Undesignated areas on the map remain unknown. (Map constructed from: Akahira and Sakagami 1959a, 1959b; Avetisyan 1960; Damus and Otis 1997; Diniz-Filho et al. 1993; Engel 1999; Fernando 1979; Fuchs et al. 1996; Hadisoessilo et al. 1995; Kapil 1956; Kshirsagar 1973, 1983; Kwon and Huh 1992; Lawrjochin 1960; Limbipichai 1990; Maa 1953; Mattu and Verma 1983a, 1983b, 1984a, 1984b; Muzaffar and Ahmad 1989; Narayanan et al. 1960a, b, 1961; Ono 1992; Otis 1991; Otis and Hadisoessilo 1990; Peng et al. 1989; Pesenko et al. 1989; Rinderer et al. 1989; Ruttner 1988, 1992; Ruttner et al. 1978, 1989; Sakai 1956, 1958; Sasaki 1994; Schneider and Djalal 1970; Schneider and Kloft 1971; Singh et al. 1990; Sylvester et al. 1998; Tilde et al. 2000; Tokuda 1924; Verma 1990, 1992; Verma et al. 1989, 1994; Zhen-Ming et al. 1992)

The analysis of the climatic zones for this botanically palaeotropical part of Eurasia shows that the whole region has a warm temperate, rainy climate with dry winters in the system of Koppen and Geiger (Muller 1982); alternatively, in the system of Troll and Paffen (cf. Muller 1982), Kashmir alone falls into the subtropical steppe while the rest of the transect from Himachal Pradesh through Nagaland is classically tropical but with varying rainfall. Physiographical changes in the southern Himalayan region are quite pronounced with respect to altitude and, indeed, increases in honeybee size are correlated with increasing altitude. This physiographic differentiation is strongly reflected in defining the boundaries of the four major morphoclusters because the seasonality of reproductive swarming varies with altitude, not longitude, in the southern Himalayan region (Table 4.1). Consequently, whichever the morphocluster region, swarming usually begins in mid-February in lowland valleys and on plains below 1,000 m. At intermediate altitudes (1,000–1,500 m), swarming commences in mid-March and at higher and more temperate levels (1,500–3,000 m), swarming is delayed until early April.

The effect of this variation for the onset of swarming is that significant temporal reproductive barriers exist between adjacent morphoclusters of *A. cerana*. For example, in the morphocluster I region 88 % of swarming begins in April while for morphocluster II 50 % of swarming begins in February/March (region 2 = 7.74, 2 degrees of freedom (d.f.), $P = 0.0208$). A combination of clusters I and II shows that 65 % of swarming begins in April while in region III 61 % occurs in February/March. Swarming in the morphocluster III region is also significantly different from that of IV (2 = 10.60, 4 d.f., $P = 0.0314$). So while there is certainly a degree of temporal overlap in the swarming periods of adjacent morphoclusters of *A. cerana*, there are also substantial and significant periods of temporal isolation between them as well. This effect has also been noted for morphoclusters of *A. mellifera* (Hepburn and Radloff 1998).

In the analysis of honeybee population structure considerable insight about genetic variability and gene flow is reflected in the variance characteristics of a particular trait. In the case of African *A. mellifera*, variance domains occur at transitions between differing ecological or climatological zones and represent zones of hybridization (Hepburn and Radloff 1998). In the case of southern Himalayan *A. cerana*, ten high variance domains occur, nearly all of which are situated at or near borders between morphoclusters, their biometric subgroups, or areas of rapid physiographic change. In the absence of complete temporal reproductive isolation, it is inferred that these high variance areas result from introgressive hybridization between adjacent morphoclusters and biometric groups. Such an interpretation could benefit from additional confirmation using DNA probes.

A final matter concerns subspecific classification of these *A. cerana* honeybees. In a recent review of this problem, it was shown that subspecific categories are riddled with anomalies and are biologically tenuous at best (Hepburn et al. 2001a, b). Previously, Engel (1999) analysed and corrected the subspecific nomenclature for *A. cerana* by rigorous application of the rules of the International Code of Zoological Nomenclature (ICZN). This was a matter of rules for names, not comment on biological entities. In any event, in his assessment the two morphoclusters representing

Kashmir and Himachal Pradesh would be *A. cerana cerana* while both morphoclusters III and IV would be the subspecies *A. cerana skorikovi*. However, all four morphoclusters obtained by multivariate morphometric analysis (Fig. 4.3; Table 4.2) enjoy equal statistical and morphometric status and all four would fully qualify as morphological subspecies of *A. cerana* just as for subspecies of *A. mellifera* (Ruttner 1988). We believe that it is too soon to assign ICZN-based names to morphoclusters (subspecies?) of *A. cerana* in general; and, for the Himalayan region, we believe that it is first necessary to characterize their honeybee neighbours in Tibet to the north, Myanmar in the east, India to the south and Pakistan and Afghanistan to the west. Moreover, the philosophical and practical basis for the subspecies concept is fraught with problems and should probably be abandoned (e.g., Wilson and Brown 1953).

To complete the tally for western Asia, the honeybees of Afghanistan and northern Pakistan would presumably show close affinities to *A. cerana cerana*. The three ecotypes proposed for Sri Lanka by Fernando (1979) apparently coincide with mainland *A. cerana indica* (Damus and Otis 1997; Fuchs et al. 1996). However, it must be noted that if the bees of Sri Lanka are indeed to be named *A. cerana indica* these 'indica' are not the same biometric 'indica' found further east in Malaysia (Damus and Otis 1997). The current picture of honeybee subspecies, biometric groups, morphoclusters, ecotypes and/or biotypes in western Asia (Fig. 4.3) can only be regarded as highly suggestive and tentative because they emanate from separate studies which are not cross-compatible.

4.2.2 Northeast Asia

Northeast Asia as defined here includes China, the Manchurian plains of the former USSR, Korea and Japan. The honeybees of the vast territory of China have been systematically investigated in a lengthy series of publications, principally by Yang and colleagues (Peng et al. 1989) who reached a number of conclusions based on analyses of honeybees from more than 1,000 localities. They proposed a series of five major biometric groups or morphoclusters as well as several ecotypes (cf. Peng et al. 1989). The same groups or races have been subsequently supported (Zhen-Ming et al. 1992). There are two aspects to this work. Firstly, there are the original results of the Yang group and secondly, some new analyses and comments on parts of the same database by Peng et al. (1989).

A survey conducted by the Chinese Bee Resource Coordination Team, from 1976 to 1983, reveals that *A. cerana* are found in all the provinces of China, except the Xinjiang Autonomous Region. The major distribution area of the genus is to the south of the Yangtze river. *A. cerana* in China can be classified into five subspecies, namely, *Apis cerana skorikovi*, *Apis cerana indica*, *Apis cerana cerana*, *Apis cerana hainanensis* and *A. cerana abaensis*. Out of these, *Apis cerana hainanensis* and *Apis cerana abaensis* are reported for the first time. These findings also suggest that *Apis cerana* subspecies can further be divided into five different biometric groups and *Apis cerana hainanensis* consists of two different biometric groups or geographic ecotypes (Yang 1990).

Table 4.2 Summary of allozyme studies of *Apis cerana* (where alleles are named according to their relative mobility, authors used *A. mellifera* standards). (Hepburn et al. 2001a, b)

Locality	Sample size	Enzymes	Polymorphism	Alleles	Frequency	Reference
Rawalpindi	12 colonies	EST	No			Nunamaker et al. 1984
Pakistan	100 bees total	MDH1	No			
Meng La, southwest Yunnan, China	100 bees	EST	No			Li et al. 1986
Japan, 9 locations	13 colonies	EST	Yes	EST ⁷³	0.96 ^a	Rozalski et al. 1996
	12–48 bees/colony			EST ⁶³	0.04	
	405 workers, 48 drones	MDH1	No	MDH1 ¹⁰⁷		
Korea	5 colonies	EST	No	MDH1 ^{fast}	0.86	Lee et al. 1986
	27–30 bees/colony	MDH1	Yes	MDH1 ^{slow}	0.14	
Korea	5 apiaries	MDH1	Yes	MDH1 ^{fast}		
	20–41 bees/apiary	ACPH, APH, EST, α-GPDH, HK, IDH, ME, ODH, PGM and XDH all monomorphic	No	MDH1 ^{slow}		Lee and Woo, 1991
Sri Lanka, 10 locations	10 colonies,	ACON2	Yes	ACON2 ¹⁰⁰	0.97–1.00 ^b	Sheppard and Berlocher 1989
	≥ 15 workers/colony	EST	Yes	ACON2 ¹¹⁴	0.03–0.00	
		ME		EST ⁸⁶	0.03–1.00	
				EST ⁵⁷	0.97–1.00	
				ME ¹¹⁰	0.03–0.00	
				ME ⁹¹	0.97–1.00	
		MDH1		MDH1 ¹⁰⁹	0.95–1.00	

Table 4.2 (continued)

Locality	Sample size	Enzymes	Polymorphism	Alleles	Frequency	Reference
		ACON1, ALDO, ARGK, G-3-PDH, α-GPDH, β-HBDH, HK, IDH, LAP, PGM and TPI all monomorphic	No	MDH1 ⁷⁵	0.05–0.00	
Bangkok, Thailand	Unspecified	EST	No	EST ⁷⁰	Most common	Gan et al. 1991
Peninsular Malaysia				EST ¹⁰⁰	Least common	
Sabah, Borneo		FUM	Yes	1 common	4 rare alleles	
South Sulawesi		α-GPDH	Yes	1 common	2 rare alleles	
Indonesia		GLDH	Yes	2 common	1 rare allele	
Luzon, Philippines		MDH	Yes		2 alleles	
		SUDH	Yes	1 common	1 or 2 rare alleles	
		APH, ACPH, 6-PGD and SHDH all monomorphic				

ACON aconitase (enzyme commission number 4.2.1.2), *ACPH* acid phosphatase (3.1.3.2), *ALDO* aldolase (4.1.2.13), *APH* alkaline phosphatase (3.1.3.1), *ARGK* arginine kinase (3.3.8.9), *EST* non-specific esterase (3.1.1.1), *FUM* fumarase (4.2.1.2), *G-3-PDH* glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12), *GLDH* glucose dehydrogenase (1.1.1.47), *α-GPDH* α-glycerophosphate dehydrogenase (1.1.1.8), *β-HBDH* β-hydroxybutyric acid dehydrogenase (1.1.1.30), *HK* hexokinase (2.7.1.1), *IDH* isocitrate dehydrogenase (1.1.1.42), *LAP* leucine amino peptidase (3.4.1.1), *MDH* cytoplasmic malate dehydrogenase (1.1.1.37), *ME* malate dehydrogenase (1.1.1.40), *ODH* octanol dehydrogenase (1.1.1.73), *6-PGD* 6-phosphogluconate dehydrogenase (1.1.1.43, 44), *PGI* phosphoglucose isomerase (5.3.1.9), *PGM* phosphoglucomutase (2.7.5.1), *SHDH* shikimate dehydrogenase (1.1.1.25), *SUDH* succinate dehydrogenase (1.3.99.1), *TPI* triose phosphate isomerase (5.3.1.1), *XDH* xanthine dehydrogenase (1.2.1.37)

^aGenotypes of queens were inferred from worker and drone genotypes, allele frequencies estimated from 13 queen genotypes

^bRange of allele frequencies found in colonies

The honeybees of peninsular Korea (Fig. 4.3, area 5) have been analysed in a series of papers by Kwon and colleagues (Kwon and Huh 1992). Basically, they studied samples from 15 localities in southern Korea and placed them all within the same biometric group. In the absence of re-analysable data, these results cannot be compared with any other Asian work. Ruttner (1988) seemed to regard these bees as morphometrically intermediate between *A. cerana cerana* of the mainland and *A. cerana japonica* in Japan.

The honeybees of the islands of Japan have been extensively analysed over the last century. It is general consensus that these bees are morphometrically completely isolated from others of the *A. cerana* complex (Damus and Otis 1997; Ruttner 1988; Sasaki 1994) as well as in terms of mitochondrial DNA (mtDNA) haplotypes (De-owanish et al. 1996). This isolation provides a convenient basis for studies of natural population structure in this branch of *A. cerana* as does the fragmentation of Japan itself into a series of islands. Two distinct morphoclusters are currently recognized, one on the islands of Kyushu, Shikoku and Honshu (bees are not native to northern Hokkaido) and another morphocluster occurring only on the small island of Tsushima in the Straits of Korea (Fig. 4.3, area 31a, b). The honeybees of each of these islands have some unique properties. For example, for southernmost Kyushu, Akahira and Sakagami (1959b) demonstrated a size cline in which the southern bees were larger than their northern counterparts. Likewise, southern bees are lighter in colour than northern ones (Tokuda 1924). Moreover, at an interlocality sampling distance of less than 100 km, inter-colonial variance was low and intra-colonial variance was high. However, the inter-colonial morphometric homogeneity in the variances of honeybees on Kyushu argues for a fairly uniform single population with continuous genetic flow among them.

4.2.3 Southeast Asia

This region extends east of longitude 98° and southwards from about latitude 20° N to Timor below the equator at 10° S. The mainland is about 1.5 million/km². With the notable exceptions of Laos, Cambodia and Vietnam; it is also that area of Asia for which most of the recent analyses of honeybees have included thorough multivariate statistical analyses as well as analyses of mtDNA and various allozymes (see the following text).

Moving southwards down to peninsular Indochina, the first study of interest concerns Thailand and Malaysia. Sylvester et al. (1998) published a comprehensive morphometric study of the honeybees of this region and unequivocally established four distinct morphoclusters (Fig. 4.3, areas 19–22) one covering most of Thailand, a second southern Thailand and continental Malaysia, a third at Phuket island and a fourth at Samui island. All four of these morphoclusters could be considered as subsets of what has previously been recognised as *A. cerana indica* (Ruttner 1988, 1992). Explanations for the distinctness of the Samui and Phuket morphoclusters

have been reasonably attributed to founder effects (Sylvester et al. 1998). The meaningfulness of the designation '*A. cerana indica*' (sometimes *A. cerana javana*) for these bees is put under considerable pressure when it is remembered that the '*A. cerana indica*' of Thailand, Borneo and Malaysia are certainly not the same bees called '*A. cerana indica*' which occur in India and Sri Lanka (Damus and Otis 1997; Fuchs et al. 1996; Ruttner 1988).

In another recent study of this region, Damus and Otis (1997) performed multivariate analyses of insular Malaysia and Indonesia and obtained four distinct morphoclusters (Fig. 4.3, areas 23–26): one isolated island cluster on Timor (area 25) (one of these in extreme southern Sulawesi in area 24 was first noted in Hadisoelilo et al. 2008). The greater part of Indonesia formed one morphocluster (Fig. 4.3, area 23) with the exception of one small cluster in southern Sulawesi (area 24) and the bees of Sabah, northeast Borneo (Fig. 4.3, area 26) yet another. In the classical literature, all of these bees belong to the *A. cerana indica* complex (Ruttner 1988, 1992), but are sometimes referred to as *A. cerana javana* (Damus and Otis 1997; Engel 1999).

Damus and Otis (1997) also included bees of the Philippines (Fig. 4.3, areas 28–30) in their study and concluded that they are morphometrically distinct from the *A. cerana indica* of Indonesia. Moreover, they found that the bees of Luzon were morphometrically distinct from those of Mindanao. Coupling their morphometric data with the mtDNA results obtained by Smith and Hagen (1997), they questioned whether the bees of Luzon actually belong to any of the *A. cerana* groups. We return to this problem in considering morphometrics, mtDNA and allozymes conjointly.

Finally, the most recently analysed island group of honeybees is that of Tilde et al. (2000) who extensively covered the Philippines using standard multivariate methods. They found three distinct morphoclusters (Fig. 4.3, areas 28–30) corresponding to Luzon island (with highland and lowland ecotypes), another morphocluster on the islands in the Visayas and Mindanao groups and a quite separate cluster on Palawan. The bees of Palawan were quite distinct from the others. All of these bees were tentatively regarded as *A. cerana philippina* by Ruttner (1988).

4.3 Mitochondrial DNA Diversity

All of the more recent studies have focused on one region of the honeybee mitochondrial genome, from the cytochrome oxidase I gene (COI) to the cytochrome oxidase II gene (COII). Between COI and COII, lie the leucine tRNA^{UUR} gene and a non-coding sequence that is apparently unique to *Apis* (Cornuet et al. 1991). The non-coding region is small in *A. florea*, *A. andreniformis* and *A. dorsata* (on the order of 24–32 bases), but larger in the cavity-nesting bees (89–97 in *A. cerana*, 94 in *A. koschevnikovi*, and ~200–900 in *A. mellifera*). Because it is non-coding, this sequence is free to evolve rapidly, and provides information analysable at the

intraspecific level. In addition to this region, Sihanuntavong et al. (1999) also examined polymerase chain reaction (PCR)-amplified fragments containing portions of the genes for the small and large subunit ribosomal RNA genes (ssRNA and lsRNA).

Groups detected by all comparative studies are: (1) mainland Asia including Japan; (2) Sundaland (including southern or peninsular Thailand and the island of Samui); (3) Palawan (Philippines); and (4) the oceanic islands of the Philippines.

The Asian mainland group contains a large number of related haplotypes. It includes samples from Japan, Korea, China (Hong Kong, Yunnan), Nepal, Vietnam (northern and southern), northern Thailand, and some of the bees from India. De-owanish et al. (1996) were also able to discriminate samples from Honshu Island, Japan, from their other mainland Asian samples by a *Hae*III restriction site polymorphism in a fragment of mtDNA including part of the leucine tRNA gene, the non-coding region and the 5' end of the COII gene. Studies using the sequence of the non-coding region alone (Smith and Hagen 1997; Smith et al. 1999, 2000) were unable to discriminate Japanese bees from the bees of Korea and other parts of the mainland (Table 4.2).

The Sundaland group of haplotypes includes samples from peninsular Thailand and Malaysia, and the islands of Samui, Phuket, Borneo, Java, Bali, Lombok, Timor and Flores as well as south Sulawesi. The islands lie on the broad Sunda continental shelf of Southeast Asia (Heaney 1985, 1986, 1991). Ken et al. (2003) studied the morphological features of *A. cerana* Fabr. in Yunnan Province of China using morphometrical methods. Samples of *A. cerana* were collected from feral colonies in 14 locations of Yunnan Province, covering the main ecological regions. The 38 standard morphometric characters were measured. The data were statistically analysed by factor analysis, discriminant analysis and cluster analysis and compared to the samples from the Oberursel data bank from Beijing, Japan, Korea, Thailand, India, Burma, Vietnam and Nepal. The results showed a high degree of variation, which correlated to geographical parameters. Bees from the northern high-altitude areas were clearly larger and darker, and showed similarity to data bank samples from Beijing, Nepal, or northern India, whereas bees from lower, southern areas clustered with bees from Thailand and Vietnam.

Smith et al. (2004) investigated genetic variation and biogeography of the cavity-nesting honey to add them to the larger picture of *A. cerana* biogeography in Asia. Non-coding regions of mtDNA of 23 colonies collected from 12 localities were sequenced to identify their genetic lineages. Six haplotypes were found (Japan1, Nepal1, ThaiS1, BurmaN1, BurmaN2 and BurmaN3) belonging to two *A. cerana* mtDNA lineages: Mainland Asian and Sundaland. The Mainland lineage was found in most parts of Burma except the Southeast, where a Sundaland population was found. Studies in Thailand suggested that the Sundaland lineage was not found north of 10°34'N; this study shows there is a Sundaland population in Burma at 19–20°N latitude. They proposed three hypotheses to explain the presence of the Sundaland lineage in Burma: (1) Burma Sundaland bees are a relict of a formerly more widespread Sundaland population; (2) Sundaland bees migrated to this part of Burma from the southern Thai-Malay peninsula; or (3) transportation by humans.

Radloff et al. (2005a) made morphometric analyses of *A. cerana* workers from 123 localities in oceanic Asia on the whole oceanic group, within specific island systems, and specific mainland-oceanic island 'interfaces'. Principal component analysis of the total oceanic database yielded two distinct morphoclusters: (1) the bees of Japan and (2) the bees of all the other islands. Discriminant and hierarchical cluster analyses showed overlapping regional clusters in the latter: 2.1 the bees of the Philippines (except Palawan) and some Indonesia, 2.2 bees of Palawan, Malaysian Borneo, Kalimantan, Sumatera and some Sulawesi (Indonesia), and 2.3 most of Indonesia, Papua New Guinea, Hainan (China) and Sri Lanka. Significant differences between the means of the four groups were demonstrated using Wilks' lambda statistic. The Mahalanobis distances among the honeybee samples are consistent with cyclical, geological rises and falls of sea level between present and Pleistocene land areas.

Radloff et al. (2005b) conducted multivariate morphometric analysis of *A. cerana* populations of the Hindu Kush in Afghanistan and Pakistan and of Kashmir and Himachal Pradesh in India and found two statistically distinct morphoclusters, a Hindu Kush/Kashmir group and a Himachal Pradesh group. High inter-colonial variances at three localities are associated with regions of major climatic zone change, hence ecological instability.

Radloff et al. (2005c) performed multivariate morphometric analyses on a series of worker honeybees, *A. cerana*, representing 557 colonies from all of southern mainland Asia extending from Afghanistan to Vietnam south of the Himalayas. They found that on the basis of scores from the principal component analysis, five statistically separable but not entirely distinct morphoclusters of bees could be identified as (1) the Hindu Kush, Kashmir, N. Myanmar, N. Vietnam and S. China; (2) Himachal Pradesh region of N. India; (3) N. India, Nepal; (4) central and S. Myanmar and Vietnam, Cambodia, Thailand, S. China and peninsular Malaysia; (5) central and S. India. The major morphoclusters are distributed coherently with the different climatic zones of the region. While populations are definable, nomenclatural adjustments remain for the future.

Xian et al. (2006) analysed 38 morphometric characters of *A. cerana*. The specimens were collected from 20 feral colonies in 8 locations in Henan, China and found that the size and colour of *A. cerana* showed significant geographical variation. In a similar study, Xian et al. (2007) studied 38 morphometric characters of *A. cerana* samples obtained from 29 colonies at 9 locations in the Jiuzhaigou-Minshan mountain zone in China and classified honeybees into 3 groups. Bees from Jiuzhaigou, which belonged to the same group, were slightly related to bees from Minxian, Lintanxian, Dingxi Pingliang and Feng'an, Gansu, and more closely related to bees from Henan and Shanxi. Bees from Jiuzhaigou-Minshan were highly diverse in terms of body colour and size. Ken et al. (2006) studied the biogeography and intraspecific variability of the eastern cavity-nesting honeybee, *A. cerana* in China using morphometrical methods together with restriction and sequence analysis of two different regions of mtDNA. Samples of *A. cerana* were collected from feral or traditionally managed colonies in 19 locations of the Chinese mainland, covering the main ecological regions. An mtDNA fragment containing a non-coding region was amplified and analysed with the restriction enzyme *Dra*I. This fragment was sequenced for two

samples. For a subset of samples, the subunit 2 of the mitochondrial nicotinamide adenine dinucleotide (NADH) gene was amplified and sequenced. Morphometric analysis revealed a high degree of variation, strongly associated with ecological zones and correlated with geographical and climatic parameters. Two main clusters were apparent, one comprised the bees from the southern tropical seasonal rain forest region, showing strong associations to the bees of Vietnam, Thailand and Myanmar. The second main cluster included the bees from the temperate deciduous broad-leaved forest region, the subtropical evergreen broad-leaved forest zone, the high, cold meadow and steppe region and the north, and showed increasing similarity to the bees of Korea and Japan. In particular, the bees from Qingzang plateau, on the fringe of the Ganshu province, were set apart by their exceptional body size, darkness and pilosity. There was no variation in *Dra*I restriction patterns within China. Sequence variation of the mitochondrial nicotinamide dehydrogenase subunit 2 (ND2) region was consistent with geographic patterns of morphological variation. Bees of the South Yunnan region were set apart by characteristically broad abdominal sterna and wax mirrors, with this locally restricted trait transcending the main transition line at the northern limit of the tropical seasonal rain forest region. This northern limit appears to correspond to the separation line between *A. cerana indica* and *A. cerana cerana*.

Ken et al. (2007a) investigated DNA sequence diversity in a non-coding portion of the mitochondrial genome in samples of *A. cerana* from 47 locations in China. Nine haplotypes (mitochondrial genotypes) were found: Japan1, Japan2, Korea4, and Cambodia2, which were previously reported from other populations, and China1–5, which are new. All nine sequences belong to the Mainland mitochondrial lineage, and none differed from the Japan1 haplotype by more than a single base substitution and/or a single insertion/deletion. Japan1 is the most common haplotype, making up 39 of 49 sequences. Haplotype diversity was 0.4 (s.d. 0.089) and nucleotide diversity was 0.00569 (s.d. 0.00154). By both measures the Chinese samples were more diverse than those from Japan and Thailand, similar to populations from Pakistan, Burma and Korea, and less diverse than samples from Indochina (Laos-Cambodia-Vietnam).

Ken et al. (2007b) found that *A. cerana* worker bees larger in size than normal can be produced both by rearing them in larger cells and also by rearing them in mixed-species colonies.

Ken et al. (2008) made multivariate statistical analyses of the morphometric characters of worker bees of *A. cerana* collected from 188 colonies at 68 localities throughout China with a sampling resolution of 1 locality/50,000 km² and found that bees from different localities form overlapping regional clusters: 1. bees from Jinlin, Liaoning, Beijing and northern Shanxi provinces; 2. larger bees from southern Ganshu and northern and central Sichuan; 3. smaller bees from southern Yunnan, Guangdong, Guangxi, Hong Kong and Hainan; 4. bees from the rest of China. Hierarchical cluster analysis using data for the China regional groups 1–4 and adjacent countries yielded a dendrogram of two main clusters. Colonies from Japan, Korea and Russia were linked with those from China regional groups 1, 2 and 4; colonies from northern Vietnam, north eastern India, Thailand and Myanmar were linked

with those from China regional group 3. *A. cerana* populations are continuous and panmictic, and morphometric structuring is indistinct.

Hadisoesilo et al. (2008) performed multivariate morphometric analyses on workers of *A. koschevnikovi* from throughout their distribution in Malaysia, Borneo and Indonesia. Principal component analysis showed one morphocluster comprising bees from Kalimantan Indonesia, Sarawak, Sabah and the Malay Peninsula. The population was more homogeneous than *A. cerana* over the same geographical area, as seen from the average coefficient of variation in 12 characters in *A. koschevnikovi* (1.8 %) compared to those same characters in *A. cerana* (4.3 %). *A. koschevnikovi* is delimited to the tropical evergreen forest regions of Sumatera, Borneo and the Malay Peninsula (Fig. 4.1). The altitudinal distributions show that *A. koschevnikovi* extends from sea level to about 1,600 m. This significantly differs from *A. nuluensis* but not *A. cerana*. It appears that the range of *A. koschevnikovi* is diminishing because it is now either poorly represented or absent in several areas where it has been previously recorded.

Jie et al. (2009) morphometrically analysed workers of *Apis cerana cerana* sampled from Longxi Mountain National Nature Reserve (LMNNR) in Fujian, China, and its peripheral region with 31 morphometric variables. Six morphometric variables were discovered with significant differences. Compared with hand-feeding *A. cerana cerana* from its peripheral region, wild *A. cerana cerana* from LMNNR had smaller longitude of forewing (8.6655 mm), longitude of wax plate (1.1568 mm) and distance between wax plates (0.2083 mm), which were decreased by 0.0728, 0.0382 and 0.0730 mm, respectively. Wing angle D7 (95.6°) became up to 1.8° larger. Wing angle N23 (83.3°) and wing angle J16 (101.2°) were decreased by 4.0 and 3.2°, respectively. The cluster analysis with discriminant function values of gravity of each sampling point resulted in two separable populations occupying ecologically different areas. This result revealed the special ecological reproduction isolation mechanism for *A. cerana cerana*, which was the reason of microevolution and provided more theoretical supports to effectively preserve indigenous *A. cerana cerana* as a genetic resource for future utilization in honeybee breeding programmes.

Radloff et al. 2010 performed multivariate morphometric analyses of *Apis cerana* Fabricius, 1,793 across its full geographical range. They found that principal component plots did not reveal distinct morphoclusters. Further substructuring of the principal component plots could not initially be derived but only by introducing local labelling did it reveal six main morphoclusters designated as 'Northern *cerana*', 'Himalayan *cerana*', 'Indian plains *cerana*', 'Indochinese *cerana*', 'Philippine *cerana*' and 'Indo-Malayan *cerana*'. *A. cerana* naturally occurs in climatic zones ranging from rainforest, savanna, steppe, grasslands and deciduous forest to taiga. The distributions of the morphoclusters are related to these physiographic and climatic factors. The taxonomy of *A. cerana* is formally revised and synonymous specific and infraspecific names summarized.

Rueppell et al. (2011) studied the nuclear microsatellite population structure in *A. cerana*, single workers of *A. cerana* colonies from Thailand genotyped at 18 microsatellite loci. The loci showed intermediate to high levels of heterozygosity and a range of allele numbers. The analyses confirmed a fundamental subdivision

of the Thai *A. cerana* population into the 'Asia Mainland' and 'Sundaland' regions at the Isthmus of Kra. However, the nuclear microsatellite differentiation was less distinct than mtDNA haplotype differences, suggesting male-biased dispersal and population admixture. Overall, samples showed a weak isolation-by-distance effect. The isolated population on Samui island was most differentiated from the other samples.

Smith (2011) summarized the current state of knowledge on mtDNA, including both coding and non-coding regions. She reported that this tool is now one of the primary sources of data used in the study of animal population biology, biogeography and phylogeny and has played a major role in the study of intra- and interspecific variation in honeybees. It is now possible to see the large framework of *A. cerana* phylogeography as indicated by mtDNA, and four main 'lineages' or groups of closely related mtDNA *A. cerana* haplotypes have been observed. mtDNA, nuclear genes and morphometrics do not always paint the same picture of *Apis* diversity and biogeography although they are in broad agreement. In-depth studies of the other Asian honeybees are yet to be completed.

Jie et al. (2011) studied the distribution and morphometric characteristics of populations of the Chinese honeybee, *Apis cerana cerana*, and the diversity and genetic characteristics of *A. cerana cerana* in different ecological regions in Fujian, South-east China, a total of 780 worker bees of *A. cerana cerana* collected from 11 samples throughout Fujian province were studied using morphometric methods. The 30 morphometric characters according to Ruttner et al. (1988) were measured. The data were statistically analysed by analysis of significance of difference, discriminant analysis and cluster analysis. The results showed that a high degree of morphometric difference existed among the honeybees in Fujian. There were at least three populations of *A. cerana cerana*, i.e. northern Fujian population, central Fujian population and southern Fujian population. The honeybees of northern Fujian had significantly larger size of body and wax mirror, longer fore wing and proboscis and special forewing angles ($P < 0.01$). The honeybees of Wuyi had the biggest wing angles G18, J10 and L13, and the smallest wing angle E9 ($P < 0.01$). The honeybees of Guangze and Zhenghe had the smaller wing angles K19, O26 and L13, and the larger wing angles B4 and N23 ($P < 0.01$). The honeybees of central Fujian population from Fuzhou, Youxi, Jiangle and Ningde differed significantly from those of other samples with larger body size, organs and the larger wing angles G18 and K19, the median N23 ($P < 0.01$). The honeybee population from southern Fujian, Longyan, Yongding, Wuping and Zhangzhou differed significantly from those of other samples with smaller size of body and organs ($P < 0.01$). Morphometric analysis of angles of forewing veins might be a useful tool for biodiversity studies of honeybees and other bees. This study provides more theoretical support for effectively preserving Fujian indigenous *A. cerana cerana* as a genetic resource for future utilization in Asian honeybee breeding programs. The islands of the Philippines are home to a diverse collection of *A. cerana* populations, belonging to at least two mitochondrial lineages: the Palawan group and the oceanic Philippine islands group. These are (1) an Asian mainland mtDNA lineage, and three additional mtDNA lineages occurring on lands connected to the mainland for successively shorter periods of time,

(2) Sundaland (connected to the mainland during the mid and late Pleistocene), (3) Palawan (connected only during the mid-Pleistocene) and (4) the oceanic islands of the Philippines (never connected to the mainland).

4.4 Overview of Subspecies and Biometric Groups through Morphometric Studies

Hepburn et al. (2001a, b) reviewed the morphometric research work on *A. cerana* in western Asia, Northeast Asia and Southern Asia earlier carried out by different investigators, namely, Akahira and Sakagami 1959a, 1959b; Avetisyan 1960; Damus and Otis 1997; Diniz-Filho et al. 1993; Engel 1999; Fernando 1979; Fuchs et al. 1996; Hadisoesilo et al. 1995; Kapil 1956; Kshirsagar 1973, 1983; Kwon and Huh 1992; Lawrjochin 1960; Limbipichai 1990; Maa 1953; Mattu and Verma 1983a, 1983b, 1984a, 1984b; Muzaffar and Ahmad 1989; Narayanan et al. 1960a, b, 1961; Ono 1992; Otis 1991; Otis and Hadisoesilo 1990; Peng et al. 1989; Pesenko et al. 1989; Rinderer et al. 1989; Ruttner 1988, 1992; Ruttner et al. 1978, 1989; Sakai 1956, 1958; Sasaki 1994; Schneider and Djalal 1970; Schneider and Kloft 1971; Singh et al. 1990; Sylvester et al. 1998; Tilde et al. 2000; Tokuda 1924; Verma 1990, 1992; Verma et al. 1989, 1994; Zhen-Ming et al. 1992.

According to these researchers, so far eight different subspecies/biometric groups of *A. cerana* have been identified each having several geographic ecotypes within them and are listed in Fig. 4.1.

mtDNA diversity in *A. cerana* has been studied by Arias and Sheppard 1996; Arias et al. 1996; de la Rua et al. 2000; Deowanish et al. 1996; Sihanuntavong et al. 1999; Smith 1991; Smith and Hagen 1997; Smith et al. 1999, 2000. According to these authors, so far only four groups have been reported. These include: Group 1 consists of mainland Asia with subgroups 1a, Japan; 1b, southern India; 1c, Himalayan region, Indochina peninsula, southeastern China and Korea, Group 2 consists of Sundaland region of peninsular Thailand, Malaysia and Indonesia, Group 3 comprises Palawan (Philippines) and Group 4 comprises the oceanic islands of the Philippines.

The greatest obstacle to a reasoned synthesis of infraspecific categories in *A. cerana* at present is that the results of most of such studies cannot be collated and unified because of fundamental and incompatible differences in statistical analyses, sample sizes, character suites, morphocluster confidence limits, the critical elements of sampling distance and extent of geographical coverage (Daly 1991, 1992; Ruttner 1988; Ruttner et al. 1978).

4.5 Superior Subspecies and Ecotypes of *Apis cerana* matching *Apis mellifera*

Factor and discriminant analysis work carried out by Verma has identified genetic variance in morphological characteristics of *A. cerana*. Such morphometric data on genetic variance is further supplemented by other biological and economic characteristics. The geographic ecotypes of *Apis cerana cerana* especially in certain

Table 4.3 Ecotypes of *Apis cerana* F. in India. (Source: Kshirsagar 1983)

Geographic region	Latitude	Altitude	Location of sample collection	Remarks
Kashmir Valley	34° 05'	1,586	Srinagar, Jammu and Kashmir	Largest ecotype in the country
Western Himalayas	31° 43'	761	Mandi, Himachal Pradesh	Possibly includes the next two variants
Western Sub-Himalayas	30° 05'	700	Kangra, Himachal Pradesh	Possibly variant of Western Himalayas
Western Sub-Himalayan Foot Hills	30° 10'	630	Ranipokhari, Uttar Pradesh	Possibly variant of Western Himalayas, and not ecotype
Eastern Himalayas	26° 53'	1,500	Kurseong, West Bengal	Verma (1992) proposes three races in this region
Indo-Gangetic Plains and Aravali Hills	29° 13'	440	Haldwani, Uttar Pradesh	Mahabaleshwar included due to its high altitude
	26° 06'	53	Muzaffarpur, Bihar	
	26° 05'	54	Guahati, Assam	
	24° 36'	1,195	Mount Abu, Rajasthan	
Central Peninsula	17° 56'	1,382	Mahabaleshwar, Maharashtra	Kodaikanal included due to its high altitude
	20° 48'	27	Cuttack, Orissa	
	17° 50'	767	Lammasingi, Andhra Pradesh	
	17° 00'	670	Petlond, Maharashtra	
Western and Eastern Ghats	15° 20'	700	Castle Rock, Karnataka	Smallest ecotype in the country
	14° 57'	700	Yellapur, Karnataka	
	12° 57'	650	Sakleshpur, Karnataka	
	10° 14'	2,343	Kodaikanal, Tamilnadu	
Western and Eastern Peninsular Coastal strips	14° 25'	0	Kumtha, Karnataka	
	11° 55'	0	Pondichery, Pondichery	
	10° 46'	97	Palghat, Kerala	
	08° 44'	51	Tirunneveli, Tamilnadu	
	08° 05'	37	Kanya Kumari, Tamilnadu	

Table 4.4 Comparison of some colony characteristics of *Apis cerana*, and of introduced temperate-zone *A. mellifera*, in India

Parameter	<i>A. cerana</i>			
	Plains	Hill	Kashmir	European <i>A. mellifera</i>
No. worker cells/cm ² (both sides of comb)	12.4	10.0	9.5	8.6
Peak adult population (thousands)	?	18–22	60–70	60–70
Relative size of colony at which swarming occurs	Small	Small	Medium	Large
Occurrence of absconding	Yes	Yes	No	No
Robbing tendency	High	High	Moderate	Low
Response to examination of combs	Strong	Strong	Slight	Slight
Response to smoke	?	Irritation	Quiet	Quiet
Honey yield	Very low	Low	High	Medium
Queenless period before laying workers develop	?	1 week	20 days	1 month
Effective defence against wasps	Yes	Yes	Yes	No
Attack by wax moths	Frequent	Frequent	None	Rare
Queen age at mating (days)	?	4–8	4–6	6–13
Introduction easy	?	Yes	Yes	No
Daily egg-laying capacity	300–500	500–800	1,400–2,000	900–1,400
Drone laying if unmated	?	Rare	Rare	Common

Table 4.5 Behaviour and performance of Kashmiri race and other hill type of Indian hive bee and the European hive bee

Character	Kashmiri race	Hill type	European bee
Egg-laying capacity of queen (range, number of eggs laid per day)	1,438–2,033	500–800	871–1,368
Population at peak of the season (number of bees)	60,000–70,000	18,000–22,000	65,000–70,000
Honey yield (range, kg/colony/year)	15–35	4–16	3.0–19.5
Pollen-carrying capacity (wt., g/bee)	0.0187	0.0140	0.0197
Homing instinct (time in seconds taken by bees to reach their hive when a frame of bees was shaken 50 m away from their hive)	180.0	191.8	295.4
Flight range (km)	3.75	1.0	3.0–4.0

Table 4.5 (continued)

Character	Kashmiri race	Hill type	European bee
Weight gain in colony per day during nectar flow (g)	6.500	1.375	Not available
Mating age of queen (range, days)	4–6	4–8	6–13
Temperature range (°C) for bee activity	Active at 8–32	Active at < 8 or > 32	Very active at 21–35
Swarming tendency	Moderate; usually after 9–12 frame strength	High; even when colony is on 4 frames	Only after colony attains 12–15 frame strength
Spring build-up	Quick	Moderate	Quick
Thriftiness	Thrifty	Slightly thrifty	Not thrifty
Queen introduction	Easy, even <i>A. mellifera</i> queens accepted	Easy, even <i>A. mellifera</i> queens accepted	Difficult, own queens also rejected, if reintroduced
Laying worker development	May develop after 30 days	Develop within a week	Not even after a month of queenlessness
Cleanliness	Good	Poor	Good
Abscending tendency	Low	High	Low
Propolis	Not collected	Not collected	Collected
Bee response during inspection	Remain calm	Agitated, run helter-skelter, not steady	Remain calm and steady
Nature of sting	Quite painful	Painful	Quite painful
Fanning position at hive entrance	Face away from hive entrance	Face away from hive entrance	Face towards hive entrance
Response to wasp attack	Defends effectively	Defends effectively	Cannot defend
Response to wax moth	Not serious	Easily affected	Rarely affected

Table 4.6 Comparative morphometric, behavioural and economic characteristics of *Apis mellifera* and *Apis cerana*. (Source: Chahal 1993)

Characteristics	<i>A. mellifera</i>	<i>A. cerana</i>
Body weight (mg)	90–120	50–70
Tongue length (mm)	5.7–7.2	4.39–5.53
Nectar load (mg)	40–80	30–40
Pollen load (mg)	12–29	7–14
Flight range (km)	2–5	0.8–2
Egg-laying capacity of queen per day	800–1,800	300–800
Colony build-up at honey flow	40,000–60,000	25,000–30,000
Swarming	Little	High tendency
Abscending	Very little	Very high tendency
Aggressiveness	Usually calm	Mostly furious
Yield under Indian conditions (kg/colony)	25–30	4–5

parts of northwest Indian Himalayas & Jumla region of Nepal match *A. mellifera*. They are the large-sized geographic ecotypes of *A. cerana* studied so far and are darker in colour than other subspecies and other ecotypes of *A. cerana* bees. Investigations carried out reveal that many of their economic and biological characteristics are quite similar to *A. mellifera* and has spectacular potential for their further genetic improvement by selective breeding and molecular research. Some of the colony characteristics of *A. cerana* matching *A. mellifera* include no. of worker cells/cm², peak adult population, relative size of colony at which swarming occurs, occurrence of absconding, robbing behaviour, response to examination of combs, response to smoke, honey yield, queenless period before laying workers develop, effective defence against wasps, attack by wax moths, queen age at mating, introduction easy, daily egg-laying capacity and drone laying if unmated (Table 4.3, 4.4, 4.5 and 4.6).

Morphometrics research data coupled with other biological and economic characteristics revealed that there may be several more economically superior subspecies/ecotypes in potential beekeeping regions of *A. cerana* which have not yet been explored and need to be investigated.

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Chapter 5

Genetics and Breeding

5.1 Introduction

Honeybee genetics is in its youth. The generation of honeybee geneticists that began their careers about 50 years ago is the first group of scientists to devote themselves exclusively to this field of study. The inspiration to study honeybees most probably came 50 years ago, as it often does today, because of the rich natural history of honeybees, and because of the excitement caused by the announcement that techniques had been developed to instrumentally inseminate queen honeybees (Watson 1927; Nolan 1929, 1932; Laidlaw 1944; Mackensen 1947; Mackensen and Roberts 1948). Instrumental insemination provided the remarkable ability to control the male parentage of a bee colony. It was the perfect complement to the longstanding methods of Doolittle (1889) which permitted the control of the source of a colony's queen. In combination, these two techniques made the study of honeybee genetics possible. For the first time, the mating of honeybees could be absolutely controlled. Other techniques previous to the development of instrumental insemination have also made the study of honeybee genetics easier. The discovery of bee space and the resulting development of the movable frame hive by Langstroth (1853) permitted the routine examination of colonies. The management of movable-frame hives for queen and drone production (reviewed by Harbo 1986) is a straightforward extension of Langstroth's contributions. All this technology development had to occur prior to the genetical study of bees. Since the technology was developed using *Apis mellifera*, most of the genetical studies of honeybees have been done with this species (Rinderer 1986). Nonetheless, some work has been done with *Apis cerana*. A review of that work will provide a starting point for a discussion of some possible new directions for work on the genetics of *A. cerana*.

5.2 Genetics of *Apis cerana*

5.2.1 Mating Biology

Apis cerana is closely related to *Apis mellifera* and displays similar behavior in many respects. Nest architecture, swarming biology, and other characteristics are all sufficiently similar that one might hope that at least infertile hybrids could be formed between the two species. Unfortunately, sufficient genetic differences exist between the species that adult hybrids cannot occur. Instrumental insemination procedures do function in crosses between the species. Spermatozoa migrate to the spermatheca, are stored there, and are used to fertilize eggs. Eggs are fertilized and, during the first 24 h, undisturbed cleavage proceeds. However, at the blastoderm stage, embryological development stops and the zygote disintegrates (Ruttner and Maul 1983). Most likely, the successful early embryonic process is driven by enzymes contributed to the egg by the queen. As these enzymes or other factors are exhausted or as other metabolic products are required for further development, the embryonic sequence is arrested. The hybrid genome does not seem able to promote the successful metabolic operations of zygote development.

Drones of *A. mellifera* may interfere with the natural mating of queens of *A. cerana* in some cases. In Germany, imported queens of *A. cerana* could not successfully mate when drones of *A. mellifera* were abundant (Ruttner et al. 1972, 1973). However, number of colonies and their relative locations, as well as number of drones, probably influence such results. Currently, beekeeping with *A. mellifera* is thriving in several parts of Asia and with locally produced and mated queens. However, at least in Thailand, the initial importation was about 100 colonies of *A. mellifera* and now several thousand colonies are in the general area. While potentially successful, the importation of *A. mellifera* has three clear dangers. First, the seriously negative experiences in South America with the Africanized *A. mellifera* from *A. m. scutellata* shows that not all stocks of *A. mellifera* are desirable for importation. Quite simply, many sources of germplasm are best left at home. Second, if breeding populations of *A. mellifera* become established and the mating of *A. cerana* is prevented because of competition by drones, or *A. cerana* is in some other way harmed, then potentially interesting and scientifically and economically useful variation in genomic material of *A. cerana* may be lost. Such loss is very serious and steps should be taken to prevent it. Third, the accidental introduction of pests and diseases is an ever-present danger. Unnoticed or unimportant symbionts of the honeybees in one area may be devastating to the honeybees of another area.

5.3 Tools for Genetical Studies

The fundamentals for doing genetical studies with *A. cerana* are available. For example, instrumental insemination technology has been successfully employed with *A. cerana* to produce inbred lines of bees (Woyke 1973, 1975, 1979). However, there

were some important adaptations that were required for successful inseminations. Only those queens inseminated with the semen of more than 15 drones laid normal number of fertilized eggs. In part, this requirement for more drones comes because drones of *A. cerana* only produce about 0.2 L of semen, one-tenth of what is common for drones of *A. mellifera*. However, the efficiency of the movement of spermatozoa from the median oviduct to the spermatheca is greater for *A. cerana* than it is for *A. mellifera* (Woyke 1975). Also, the spermathecae of queens of *A. cerana* have far fewer spermatozoa after natural mating than do the spermathecae of queens of *A. mellifera* (Woyke 1975). Thus, although a greater number of drones is required for the successful instrumental insemination of queens of *A. cerana*, the number is less than what might be predicted from some of the data.

After observing the process of instrumental insemination of queens of *A. cerana* in Thailand, I can offer several suggestions to those new to the techniques. If possible, the techniques should be first perfected with *A. mellifera*. Small queens of *A. mellifera* can be difficult to inseminate. In the initial development of instrumental insemination, Watson discarded a stock of queens as unsuitable because of their small size. The technique is best learned with larger queens. If queens of *A. mellifera* are not available, then at least larger *A. cerana* can be used. The entire process of insemination involves the collection of semen and then its insertion into the median oviduct of the queen. In order to facilitate learning, it is useful to learn each part of the process separately. Collecting semen is an art in itself and is best learned as a separate task. The successful insertion of semen into the median oviduct of a queen can also be learned separately. Cow's milk or similar material can be used instead of semen and insemination successes can be inferred if little or no fluid leaks from the queen as it is injected. A complete description of the insemination process is available in English along with excellent photographs and drawings (Harbo 1986). The management techniques of queen rearing and drone production are apparently rather straightforward adaptations of these techniques as they are employed with *A. mellifera*. Good descriptions of these management techniques have been provided by Harbo (1986). However, attention should be paid to specific requirements of bees in different climates and ecosystems.

Generally speaking, the use of instrumental insemination is best reserved for specific genetic studies. Its use in bee stock improvement programs has limited practical value. The costs of a breeding program based on instrumental insemination are usually prohibitive. Breeding programs are still able to have the use of reasonable control of mating. The use of island mating stations or the mainland use of drone flooding techniques combined with reductions in the populations of unmanaged drones in mating areas are often better alternatives for controlling mating in breeding programs.

5.4 Cytogenetics

The first important study of the genetics of *A. cerana* was the description of its karyotype (Deodikar et al. 1959). The chromosome number of $N = 16$ has since been confirmed by other investigators (Sharma et al. 1961; Deodikar et al. 1980; Hoshiba

et al. 1981). The chromosome number of $N = 16$ is the same for all species of the genus (Fahrenhorst 1977). Also, one large metacentric chromosome is common to all species of the genus. These commonalities indicate that radiating evolution within the genus has been the result of events different from simple polyploid formation (Moritz 1986). Cytogenetic work with honeybees has been proved to be difficult. In all cases, chromosomes are quite small and no tissues containing polytene chromosomes have been discovered. It may be that a search of various tissues will yield sources of polytene material as has occurred with several other nondipteran insects.

5.5 Sex Determination

Sex determination in *A. cerana* was studied by Woyke (1973, 1975, 1979). He had substantial prior experience with studying the sex determination of *A. mellifera* (Woyke 1986) and consequently he tested the hypothesis that sex determination was genetically controlled in both species in similar ways. By producing inbred lines of bees, he created the possibility that the brood of the inbred queens would have a percentage of inviable eggs or larvae. This indeed was the case and he hypothesized that there are multiple alleles (Xa, Xb, XC, etc.) at the sex locus (X) males develop from unfertilized eggs (all of which are hemizygotes at the X locus) or from fertilized eggs which are homozygous at the X locus. For example, a queen inseminated by one drone (XaXb x XC) produces haploid drones xa and Xb from unfertilized eggs and diploid females XaXC from fertilized eggs. If one of these daughters (XaXC) is inseminated by her brother, Xa, then this daughter will produce two types of fertilized eggs: normal heterozygotes (XaXC) from which females will develop and homozygotes (X2Xa) from which diploid males will develop. Similar progeny ratios were observed by Hoshiba et al. (1981) who confirmed the hypothesis of Woyke for sex determination of *A. cerana*. The issue of diploid drones is an interesting one. In *A. mellifera*, these newly hatched larval drones are eaten by worker bees (Woyke 1963). It is thought that they may produce sufficient quantities of a substance called “cannibalism substance” which marks them and stimulates workers to eat them (Woyke 1967; Dietz 1980). With *A. cerana*, diploid drone larvae are not eaten by the workers just after hatching but are sometimes reared to the age of 4 days. Presumably, drones of *A. cerana* also produce cannibalism substance but the quantities or timing of production are different. Hopefully, further work will clarify these issues.

5.6 DNA Studies

A few studies have examined DNA of *A. cerana*, the principal molecule of heredity. Jordan and Brosemer (1974) examined the DNA from three species of *Apis* (*mellifera*, *cerana*, and *florea*). First, the buoyant densities were determined for double stranded DNA in cesium chloride gradients. The DNA peak of *A. florea* was regular and narrow. However, the DNA peaks of *A. mellifera* and *A. cerana* were broad

and rather asymmetrical. Both showed a low guanine–cytosine content but this was especially so for *A. cerana*. Generally, these results indicate that the *Apis* species have an unusually heterogeneous base pair composition compared to other animals. Jordan and Brosemer (1974) also studied the reassociation kinetics of the DNA of *A. mellifera* and *A. cerana*. These experiments showed that these honeybees have very little repetitious DNA—only about 3–5 % of the genome. An amazingly high 89 % of these honeybee genomes is composed of unique DNA sequences. This result is especially curious when contrasted with the results of Sperlin et al. (1975). They found that only about 60 % of the DNA of *A. mellifera* forms heterochromatin with DNA of *A. cerana*. This divergence is truly remarkable in a sister-species system where the two species are so similar and where so little of the DNA is repetitious. Further investigations in these areas are bound to reveal interesting facts.

5.7 Biogeography

The range of *A. cerana* is quite large with a distribution that is allopatric to that of *A. mellifera*. This range encompasses a broad diversity of ecological conditions. In some portions of this range, the biotype or subspecies variations of *A. cerana* have received scientific attention and have been described, especially for morphological characteristics (Ruttner 1987). However, there is clearly more to do to fully document the morphological variation of *A. cerana* throughout its range. Even more importantly, the behavioural variation which is bound to exist in a species so widely distributed has been studied very little in controlled comparative experiments. This is not surprising since such behavioral investigations are far more difficult and expensive than morphometric studies. Indeed, only in Europe, a small portion of the range of *A. mellifera*, has sufficient work been accomplished to fully describe the variation of the local *A. mellifera*. Naturally occurring behavioral variation in all the honeybees in the remainder of the world has received very little scientific attention. This undescribed variation will be a chief source of scientifically and economically important questions for future generations of geneticists.

5.7.1 Examples from *Apis mellifera*

Beyond the work already cited, little is known about the genetics of *A. cerana*. However, several examples of possible areas of genetical work with the Eastern hive bee come from work already done with its Western sister species.

5.7.1.1 Classical Genetical Studies

Several visible mutations have been described and studied in *A. mellifera*. Most of them are mutations which interfere with normal pigment development which leads to

the typical black or brown eye color of honeybees. Other mutants involve the cuticular color, the presence or absence of body hair, or the morphology of the eyes, wings, and sting. Most of these mutants are recessive gene forms, but their transmission and interactions can be studied using instrumental insemination as a basic tool. The vast majority of mutants of *A. mellifera* have been discovered in drone honeybees (Tucker 1986). The sex determination system which causes naturally occurring drones to be haploid is very fortunate for those interested in finding mutants. The haploid drones display recessive as dominant ones. Certain such mutants are to be found in *A. cerana*. When they are, the classical studies of mode of inheritance, dominance, allelism, linkage, viability, and penetrance all can be conducted.

5.7.1.2 Behavioral Genetics

Behavioral genetic studies in *A. cerana* await clear behavioral descriptions. As differences between stocks or colonies are brought to light, it will become difficult for honeybee biologists to avoid wanting to explore the genetic source of these differences. There is already a strong beginning in behavioral genetics with *A. mellifera*. The classic work of Rothenbuhler (1964) is widely cited in the larger field of behavioral genetics. This work investigated the variation between stocks in their tendency to clean their nests of dead brood, a trait called hygienic behavior. Using a special genetical approach called the inbred line—single drone insemination technique in a backcross experiment, Rothenbuhler created colonies having worker bees all having the same genotype. With these techniques, genetic segregation could be studied at the colony behavior level. In this way, Rothenbuhler showed that hygienic behavior “depends upon homozygosity for two recessive genes.” Many other examples of behavioral genetic studies in honeybees exist (Rinderer and Collins 1986). Most students of honeybees are attracted to them because of their rich and diverse behavior. Because of this interest, many other examples of behavioral genetic studies will be forthcoming. Not all such work requires the special insemination procedures used by Rothenbuhler (Rinderer and Collins 1986), so many studies can already be undertaken. However, it is possible that one day someone will learn how to apply the single drone insemination technique to *A. cerana*.

5.7.1.3 Biochemical Genetics

The most striking thing about the biochemical genetics of honeybees and other Hymenoptera is the lack of variation which is found when their allozymes are analyzed. This is equally true for studies with *A. cerana*. Tanabe et al. (1970) found that esterases of *A. mellifera* and *A. cerana* were different but did not find differences between groups of *A. cerana* from three different locations. This result suggests that allozyme studies will probably not prove especially useful throughout the genus *Apis*. Since other characteristics, including DNA characteristics, do vary, it will probably be more productive to focus efforts in other areas.

5.8 Quantitative Genetics and Honeybee Breeding

Quantitative traits usually involve many genes, each contributing a small effect. In some cases, there are modifier genes at other loci which have indirect, or pleiotropic, effects on the genes directly affecting the character. There is no difference in basic chromosomal mechanics for these two types of genes, although in some cases the genes involved in controlling a continuous character may be rather closely associated on a chromosome (Falconer 1981). Such groups of many genes affecting a single character are called polygenes.

Many of the characters of honeybees that have economic importance are quantitative. To aid in more clearly defining the underlying genetic complexity, the visible expression of characters, the phenotype, must be clearly described. Some traits, such as morphological ones, are easily measured. Physiological traits, such as hormone or pheromone levels and disease resistance, may require more complex assessment. Behavioral traits, such as pollen collection and honey production, may require the development of a measurement system that divides the complex behavior into smaller, more easily studied parts. One of the basic objectives of quantitative genetics is predicting the outcome of a selection or breeding program based on observations of existing populations. Measurements are made on groups of relatives which are used to predict how future offspring will express a character. The process uses estimates of population parameters such as means, variances, and covariances.

The statistical tools of quantitative genetics are well worth having before embarking upon programs to select improved stock. The genetic potential for change in populations of *A. mellifera* is amazing. Even after long-term culturally based artificial selection in Europe, the bees of Europe respond to selection for economic traits very quickly and very well (Kulincevic 1986). *A. cerana* has been under less artificial selection in Asia, in part because of its tendency to abscond. However, there are suitable examples of colonies of *A. cerana* that do not abscond, even in conditions that trigger the absconding response in other colonies. The potential for stock improvement programs using selective breeding of *A. cerana* is tremendous. The possibilities of using native bees, selected for improved economic performance, should not be overlooked in a rush to import mite-susceptible *A. mellifera*.

5.9 Biotechnological Potential of *Apis cerana*

Recent advances in genetics and related disciplines have made it possible to move from traditional methods of controlling genetic change in organisms, such as controlled breeding programs and selection, to the ability to directly manipulate the DNA, the genetic code itself. One major focus is on the area of biotechnology known as recombinant-DNA technology, locating or assembling specific genes and placing them in the chromosomes of an organism. Also genes that appear to have the same function can be obtained from different organisms, and the sequence of the nucleotides, the building blocks of DNA, may be compared in the finest detail to

analyze what differences are present. This technology is best developed in the fruit (or vinegar) fly, *Drosophila melanogaster*, in which known genes have been placed in chromosomes and are stably inherited.

Methods of inserting genes into chromosomes are presently the major block to the application of the technology developed with *Drosophila* to other insects. Many efforts to apply *Drosophila* gene transfer technology to other insects have failed to demonstrate stable gene transfer on a repeatable basis. Most efforts have been completely unsuccessful. Therefore, the original hopes of simple technology transfer have been dashed. Efforts are now being concentrated on developing the technology of manipulating genes. This involves techniques such as locating specific genes, assembling new or modified genes from existing or synthesized parts, and alternative methods of gene transfer. While our ability to apply these new methods is mainly limited to *Drosophila*, some plants and some lower organisms, there is very active research in progress to learn how to apply similar methods of genetic manipulation to other organisms. One of those organisms is the honeybee. The present research is limited to *A. mellifera*, but preliminary research is underway to expand this research effort to *A. cerana*.

5.10 Defense of Asiatic Honeybee against Pathogens

Honeybees defend against many pathogens by producing antibiotic substances such as propolis and royal jelly (Souza et al. 2007; Fontana et al. 2004). The innate immune system is the first line of defense against pathogens in plants and invertebrate animals, and it is also critical for vertebrate immunity before the acquired immune system generates a specific response (Girardin et al. 2002; Loker et al. 2004; Müller et al. 2008; Boman 1995). Various antimicrobial peptides are the key elements of the insect immune system (Bulet et al. 1999; Hoffmann et al. 1999; Casteels et al. 1989). After the honeybees are infected by pathogens, four antimicrobial peptide families are synthesized, representing a broad spectrum of antimicrobial activity in the hemolymph. All of these are cationic peptides identified as: apidaecins (Casteels et al. 1990), abaecin (Casteels et al. 1993), hymenoptaecin (Casteels et al. 1993) and defensin (Casteels et al. 1994). Recently, two structurally different defensin genes were cloned from *A. mellifera* (Klaudiny et al. 2005). Almost all of the honeybee antimicrobial peptides and the antimicrobial peptide genes are reported from the Western honeybee; however, little research has been conducted on the Asiatic honeybee. Xu et al. (2009) designed primers for the sequences of the four antimicrobial peptide cDNA gene families (*abaecin*, *defensin*, *apidaecin*, and *hymenoptaecin*) of the Western honeybee, *Apis mellifera* L. and identified all the antimicrobial peptide cDNA genes in the Asiatic honeybee for the first time. All the sequences were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR). In all, 29 different *defensin* cDNA genes coding 7 different defensin peptides, 11 different *abaecin* cDNA genes coding 2 different abaecin peptides, 13 different *apidaecin* cDNA genes coding 4 apidaecin peptides, and 34 different *hymenoptaecin* cDNA

genes coding 13 different hymenoptaecin peptides were cloned and identified from the Asiatic honeybee adult workers. Detailed comparison of these four antimicrobial peptide gene families with those of the Western honeybee revealed that there are many similarities in the quantity and amino acid components of peptides in the abaecin, defensin, and apidaecin families, while many more hymenoptaecin peptides are found in the Asiatic honeybee than those in the Western honeybee (13 versus 1). The results indicated that the Asiatic honeybee adult generated more variable antimicrobial peptides, especially hymenoptaecin peptides than the Western honeybee when stimulated by pathogens or injury. This suggests that, compared to the Western honeybee that has a longer history of domestication, selection on the Asiatic honeybee has favored the generation of more variable antimicrobial peptides as protection against pathogens.

5.11 Present Research with Honeybees

Biotechnology-related research with honeybees is presently being conducted by the USDA, Agricultural Research Service, Honey-Bee Breeding, Genetics, and Physiological Laboratory at Baton Rouge, Louisiana. The goals of this cooperative research are to develop the technology to locate, manipulate, and transfer genes in honeybees. The specific genes may very well not be originally from honeybees. In fact, they might be at least partially synthetic genes created in a laboratory.

Research is also being conducted to identify desirable genes or parts of genes to transfer. The first gene to transfer will be one which can be identified easily so that verification of a successful transfer is simplified. Once a reliable transfer method has been developed, other genes that will result in an improved stock of honeybees can be transferred. One class of genes that is being evaluated as the most promising for improving honeybees are the genes coding for proteins known as cecropins. These genes are from insects and produce proteins that have very strong bactericidal and fungicidal effects, even at low concentrations. Thus, by transferring a single gene into honeybees, resistance to American foul brood (AFB), European foul brood (EFB), and chalkbrood might all be obtained simultaneously. Preliminary research at Baton Rouge has already shown that one cecropin is bactericidal to AFB with an LD50 of about 1 to 5 micromolar. The effects of this cecropin on EFB and chalkbrood are now being investigated. It is possible that honeybees already contain cecropins but that in some cases they are induced or "turned on" too late (after a larva is infected) to provide acceptable levels of disease resistance. Therefore, we are examining honeybee DNA to determine whether there is a sequence similar to that of this particular cecropin. If a cecropin-like gene is found in bees, it might be possible to modify the expression of this gene in honeybee larvae so that the cecropin protein is present to respond to the challenge of various diseases. It should be equally possible to conduct this transformation with *A. cerana* as with *A. mellifera*, if DNA laboratory facilities are available at a location where *A. cerana* is available.

Another area of biotechnology-related research which is beginning is that of mite resistance. *A. cerana* is the natural host of the parasitic mite *Varroa jacobsoni*. In some

way it is resistant to the effects of this mite to the extent that it can keep levels of infestation of this mite very low with no assistance from beekeepers. Having coevolved with these parasites, the Asiatic honeybee exhibits more careful grooming behavior than the Western honeybee, and appears to have other more effective defenses against these parasites. As an alternative to chemical and management methods of mite control, it would be desirable to have the same resistance in *A. mellifera* as is present in *A. cerana*. Since these two species do not hybridize, the use of recombinant-DNA methods is the only feasible way to transfer this resistance. However, in order to make this transfer, the mechanism of resistance must be identified, its genetic basis determined, the specific controlling gene(s) located, the desired gene removed, and this gene transferred. These will all be time-consuming steps, so it is important that research begin and be pursued so that the feasibility of this approach can be determined. Even if DNA transfer is not feasible or takes a very long time, the results of research into the mechanism of mite resistance in *A. cerana* will yield useful information. Knowledge of behavioral or physiological responses contributing to mite resistance in *A. cerana* may very well lead to identification of similar characteristics in *A. mellifera* and permit more efficient selection to increase their effect.

5.12 Recombinant-DNA Procedures

Specific procedures must be determined for each organism and, often, even for each laboratory, due to variations in enzymes, proteins, etc., which must be removed, chemicals used, and equipment used. Therefore, no specific procedures for bees can reasonably be presented yet. Specific protocols will also tend to change rapidly as research yields improved procedures. However, procedures which have been found to be effective in other organisms are available in laboratory manuals, including Maniatis et al. (1982) and Berger and Kimmel (1987). Berger and Kimmel's manual also includes the section "Requirements for a Molecular Biology Laboratory," which discusses equipment and procedures.

The general procedure is basically as follows:

5.12.1 DNA Extraction

A suitable tissue or life stage is determined (whole larvae or testes of drone pupae are both used for bees). The tissue or organism is ground in an appropriate buffer solution. Various chemicals are added to disrupt the cells and remove the other components of the cells (e.g., membranes, fats, proteins, enzymes that destroy DNA and RNA). This often occurs in a two-phase system (water and hydrocarbon), or through centrifugation. The purified DNA can then be used in several ways.

5.12.2 DNA Fractionation

Enzymes (restriction enzymes) are available which cut the DNA at specific places (restriction sites, which are short specific sequences of component nucleotides).

The pieces of DNA (restriction fragments) can then be separated by their size in a procedure called electrophoresis.

5.12.3 DNA Hybridization

The fractionated DNA can then be transferred to a treated membrane and fixed in place. Under proper conditions, known DNA (a probe) can be applied to the membrane. If any areas of the fractionated DNA are sufficiently similar to the probe, the DNA molecules will align and pair (hybridize). If the probe has been treated with a suitable marker before hybridization, the location of any similar fractionated DNA, if present, can be detected. By varying the conditions of hybridization, the degree of similarity can be estimated.

5.12.4 DNA Cloning

Once the location on a membrane or in a gel of a particular piece of DNA (usually a gene) has been determined, it can be separated from the rest of the DNA pieces. To increase the available amount of a particular gene, the gene can be inserted into the DNA of one of various microorganisms, and it will then be reproduced (cloned) as the microorganism reproduces. The total DNA can then be extracted and the desired gene removed using restriction enzymes. The desired gene can be increased to any quantity by controlling the reproduction of the microorganism.

5.12.5 DNA Library

If no similar gene is available or if changes have been too great for hybridization to occur, “all” of the DNA pieces produced by a restriction enzyme acting on the DNA from the organism can be cloned. This produces a great number of different clones (groups of microorganisms containing the same piece of cloned DNA) which together are called a “DNA library.” Then the clones are examined for some characteristic (screened).

5.12.6 Gene Construction

Successful expression of a gene in a foreign organism depends on two factors. First, the gene of interest must be inserted. Second, the accompanying DNA regions that control production of the gene’s product must be present and work in the new organism. These controlling elements are often quite specific in their activity. Many studies have demonstrated that the best expression is derived from a combination of the gene of interest with controlling elements from the organism to be transformed. The technology is already available to construct such a gene, if the structural gene

and control elements can be identified. The procedure is basically the same as for cloning a gene, except that two or more pieces of DNA must be inserted.

5.12.7 Vectors

After the desired gene has been identified in organisms to be transformed. Various methods have been used or proposed, with varying rates of success in different organisms. One method in use with *Drosophila* uses a particular piece of DNA (P-element) with the capability to actively insert itself into *Drosophila* DNA rather than depending on some type of passive incorporation. A piece of DNA which can actively insert itself into host DNA is called a vector. If available, use of a vector is much more efficient than passive incorporation. In *Drosophila*, a modified P-element, with the desired gene inserted in the P-element, can introduce the gene into a chromosome. Insertion of the gene into a vector is basically the same process as cloning a gene.

5.12.8 Transformation

Transformation is the stable integration of the gene of interest into the host's chromosomes, with subsequent production of the gene product. In *Drosophila*, this is accomplished by injecting a solution containing the vector, with the desired gene incorporated, into an egg of suitable age. This requires a micromanipulator, a high quality dissecting microscope, a very fine pointed syringe containing the DNA vector in solution, and appropriate treatment of the egg. In a few percent of the injected eggs, the vector will successfully integrate into a chromosome. However, if it integrates within a required gene, it will probably inactivate that gene and kill or be detrimental to those eggs. Therefore, only some of the eggs where the vector has successfully integrated will be viable. Unfortunately, no experiments to date have been able to repeatedly demonstrate successful use of the *Drosophila* P-element for transformation in any insect outside a small group of species in the Diptera. Even within the Diptera, the success rate quickly falls to zero in more distantly related species. Therefore, P-elements are unlikely to be usable in bees. Hopefully, another vector will be located. Viruses appear to be the most likely alternative, particularly the DNA viruses. Unfortunately, no DNA viruses have been reported from bees.

5.12.9 Propagation

The treated individuals must then be propagated to select transformants and to obtain the beneficial results of the insertion of the foreign gene. Compared to *Drosophila*, raising the treated individuals will probably be much more difficult in a social insect such as bees, since the workers may be able to identify treated individuals and may reject them. However, a successfully transformed queen bee will be able to produce many more offsprings over a much longer period of time than is possible in *Drosophila*.

5.12.10 Selection of Transformants

Individuals that were successfully transformed must be separated from those that were not transformed. Occasionally, this can be done by examining or testing (screening) those individuals which were produced from the treated eggs. Usually, however, treated individuals contain both transformed and untransformed germ cells. Therefore, they must reproduce to produce offspring with the gene of interest distributed homogeneously throughout their body. Screening the offspring allows selection of offspring of parents that were transformed.

Because the technology is still not developed, particularly the availability of vectors, recombinant-DNA cannot yet be applied to bees. However, it probably will not be very long until the technology is available. It is therefore important to begin to consider potential uses. More uses are apparent for *A. mellifera* because problems, such as mites and diseases, and possible solutions, such as transfer of mite resistance from *A. cerana* and transformation for the cecropin gene, have already been identified. Also, *A. mellifera* is much more important economically and so more resources are available to conduct research.

In *A. cerana*, transformation for the cecropin gene is an obvious choice. However, an important problem for some *A. cerana* beekeepers is a virus disease, the so-called "Thai sacbrood." A disease caused by a virus probably would not be affected by the cecropin protein. Identification of a gene in *A. mellifera* which conferred resistance to this sacbrood would then provide a gene which would be important to *A. cerana* beekeepers and would only be available through recombinant-DNA technology. Location of such a gene (if it even exists or is a single gene) would undoubtedly be a major undertaking with no guarantee of success, which means it may not even be attempted. Many of the characteristics of *A. cerana* such as absconding, swarming, and honey production, which need improvement to achieve the relative economic level found in *A. mellifera*, are behavioral characteristics which will not lend themselves to solutions at the DNA level. Attention therefore needs to be directed to characteristics of *A. cerana* which are probably under simpler genetic control and thus are more amenable to modification at the DNA level.

A. cerana has significant potential to be improved by recombinant-DNA technology and to contribute to the improvement of *A. mellifera*. However, its potential can only be fulfilled if those who are working with *A. cerana* are made aware of the uses and limits of recombinant-DNA technology and expend the effort to determine where DNA-level improvements could be made.

5.13 Conclusion

The potential of *A. cerana* as an organism to use in genetical studies and biotechnological manipulations is largely untapped. However, the tools exist to study the genetics of *A. cerana* in much the same way that the genetics of *A. mellifera* has

been studied. In addition, the potential for the involvement of *A. cerana* as an economically valuable organism is large and mostly unexploited. Selection programs should yield considerable success with this species in reasonably short periods of time.

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Chapter 6

Reproductive Biology

6.1 Introduction

Honeybees have haplo-diploid sex determination system, which depends on a single sex locus and inbreeding and has a severe negative impact on the viability of the offspring. Thus, natural selection favoured several behavioural mechanisms to prevent mating among related drones and queens. Social insects, and in particular honeybees have evolved a complex division of labour which resulted in an amazing individual diversity. The honeybee worker at the cost of forfeiting its reproductive abilities is highly adapted and shaped for its functions, within the colony and for foraging. On the other hand, neither the queen nor the drone is able to forage or take part in any social activity. They have been specialized for their reproductive functions. The highly developed honeybee society has shaped the behaviour and physiology of queens and drones, which resulted in several unique, dramatic and even bizarre events. At present, there are nine species of honeybees but the complete and general account on honeybee mating and reproduction has been best studied for the Western honeybee *Apis mellifera*. However, the information on reproductive activities of Asian honeybee *Apis cerana* is relatively less known.

In social insect societies, only some of the individuals reproduce. Food resources are allocated to either adult workers or sexual offspring (Seeley 1985; Hölldobler and Wilson 1990). In colonies of the highly eusocial honeybees (Apini), drones and virgin queens are produced as sexuals. Colonies allocate their resources in such a way that the chances for sexuals to contribute to the next generation are the highest. This contribution differs between drones and queens. Drones only need to search a virgin queen during her nuptial flight to mate with, whereas queens need a colony of workers to become successful (Velthuis 1990). Therefore, the production of a successful young queen implies the production of a swarm or supersedure, where the old queen leaves with a part of the bees and a young queen takes over the original colony. The size of the swarm and the moment it leaves the mother colony are the two important factors determining the latter success of the swarm and of the original colony. Hence, the number of successful young queens that a colony can produce is limited. In contrast, the number of drones produced can be very large because production costs are low and the more drones a colony can produce, the

greater the possibility that some of them will mate with a virgin queen (Velthuis 1990). To promote out-breeding, honeybees have developed a mating system in which insemination of the virgin queen occurs at drone congregation areas far from the colony, where drones from widely dispersed colonies congregate (Fletcher and Ross 1985; Punchihewa et al. 1990a, b; Koeniger and Koeniger 2000).

In Western honeybees, *Apis mellifera*, the production of drones and queens is governed by a combination of external and internal factors, which has been comprehensively studied (Winston and Taylor 1980; Ruttner 1985; Winston 1987). Much is known from *A. mellifera* colonies in temperate regions where flowering is present during a limited period of the year, which results in a defined seasonal appearance of sexuals and colony multiplication (Seeley 1985; Winston 1987). In contrast, tropical florescence is usually available over the entire year, potentially allowing the colonies to produce sexuals all year round (Allsopp and Hepburn 1997; Hepburn and Radloff 1998). The number of sexuals produced and the number of swarms issued mainly depends on food resource availability and the number of workers (Winston and Taylor 1980; Winston 1987, 1990; Allsopp and Hepburn 1997). Moreover, other factors such as fecundity and age of the queen and genetic disposition are involved (Ruttner 1983, 1985). Mechanisms and factors regulating the production of drones and queens may be similar (Winston 1987).

Currently, the honeybees (Apini) are considered to have nine species (Otis 1997) that form a monophyletic group (Ruttner 1988). Of these, *A. mellifera*, *A. cerana* (Ruttner 1988), *A. koschevnikovi* (Tingek et al. 1988), *A. nigrocincta* (Hadisoesilo and Otis 1996) and *A. nuluensis* (Tingek et al. 1996) form the cavity-nesting group of *Apis*. Bees in this group are probably similar in colony organization (Dyer and Seeley 1991). Based on morphometric variation and geographic distribution, the Asiatic honeybee species, *A. cerana*, has also been classified into four subspecies: *A. cerana cerana* in northern Asia, *A. cerana indica* in southern Asia, *A. cerana japonica* in Japan and *A. cerana himalayana* in the Himalayan region (Ruttner 1988). Beekeeping with *A. cerana* is an ancient activity. In many natural and agricultural eco-systems *A. cerana* is the most important pollinator (Verma 1990; Crane 1991; Kevan 1995). Since this species is distributed over a wide range of natural habitats and climates, in a vast geographic area of Asia ranging from Iran to China and from Japan to the south of Indonesia (Smith and Hagen 1996), *A. cerana* is expected to express basic differences in colony growth and reproduction in response to environmental differences. For example, the reproductive season is confined to 3 months in spring in the temperate region of Japan (Okada 1970; Matsuura and Sakagami 1973). In the tropical monsoon region of Northern Thailand, Southern India and Northern Pakistan, colony reproduction usually occurs from March to October (Koeniger 1976; Seeley et al. 1982; others reviewed in Seeley 1985; Ruttner 1988; Roubik 1989) whereas, no seasonality in colony reproduction has been found in the tropical rain forest of Sumatra (Inoue et al. 1990).

Although, reproduction has been widely studied in *A. mellifera*, relatively little work has been done on the sister species *A. cerana* (Koeniger and Koeniger 1991). For *A. cerana*, much of the available data focus on mating behaviour, physiology and reproductive isolation (Koeniger and Koeniger 2000). Little information exists

on seasonal cycles of colonial development, production of sexuals, swarming and supersedure. Mechanisms and factors regulating these processes are important not only for insight into the evolution of these social bees, but also for application in the breeding programs of the Asiatic honey bees in many Asian countries (Verma 1990).

Chinh et al. (2005) found that, with forage available all year round, production of sexuals (i.e., drones and queens) was restricted to two periods from March to July and from September to December. Most swarming occurred in May when forage was most abundant. Positive correlations between available forage, colony growth and production of sexuals suggest that the synchronized production of drones and queens is defined by the forage flow into the colony. If this flow is high, the colony starts growing; when the colony is large enough drones and queens are produced, and eventually the colony swarms. Production of sexuals is synchronized because foraging conditions are sufficient to allow growth for only a part of the year. Patterns in drone and queen rearing by *A. cerana* are similar to patterns found in *Apis mellifera*. Variation probably reflects differences in environment rather than differences between species.

The synchronized production of sexual offspring has also been found for *A. cerana* in other regions, both temperate (Okada 1970; Masuura and Sakagami 1973) and tropical (Sharma 1969; Seeley et al. 1982; others reviewed in Seeley 1985; Ruttnier 1988; Roubik 1989). In addition, the pattern is rather similar to what has been described for *A. mellifera* in both temperate (Seeley 1985; Winston 1987) and tropical regions (McNally and Schneider 1994; Hepburn and Radloff 1995; Otis 1990; Allsopp and Hepburn 1997; Hepburn and Radloff 1998). The correlations between flowering intensity, colony growth and production of drones and queens suggest that the synchronized production of drones and queens is defined by the forage flow into the colony. If the flow is high, the colony starts growing, then drones and queens are produced and eventually the colony swarms. If foraging conditions were good during much longer periods, honeybees would swarm more often (up to 12 swarms and after swarms per year) and the synchronized production of sexuals would be lost (Winston 1990; Otis 1990).

A. mellifera and *A. cerana* are sister species with similar traits of social structure and behaviour (Seeley 1985; Dyer and Seeley 1991). Both species have evolved to exploit flower patches for maintaining their colonies and subsequently to invest as much as possible in production of sexuals (Seeley 1985; Velthuis 1990). Therefore, it is not surprising that the general pattern of seasonality in colony growth and production of sexuals is similar between *A. cerana* and *A. mellifera*. Variation may result from the different environment the bees live in, rather than from fundamental differences between the species. For example, in *A. mellifera*, temperate-evolved bees invest a lower proportion of drone comb at relatively small colony sizes than tropical-evolved bees, but more drone comb in larger colonies (Lee and Winston 1985; Winston 1987; Winston 1990). In agreement with previous studies on *A. cerana* (Inoue et al. 1990) and *A. mellifera* (Lee and Winston 1985; Winston 1990; Allsopp and Hepburn 1997), colony size affects production of sexuals. Colonies initiate drone and queen rearing when their population is larger than 10,000 bees, which is similar to earlier studies of *A. cerana indica* (Sharma 1969; Inoue et al. 1990) and *A. mellifera* (Free and Williams 1975; Lee and Winston 1985). Chinh

et al. (2005) in their study found that the sex investment ratio was 554 males to 1 queen, the same as found before in *A. cerana indica* (556:1, Inoue et al. 1990). In *A. mellifera* similar ratios have been reported (750–833:1, reviewed by Seeley 1985; Winston 1987; Roubik 1989). Colony multiplication in honeybees takes place through swarming. Queens are produced in a small number and appropriate to the needs of the colony, while 100–1,000 times more drones are produced. This ratio probably reflects the relative costs that a colony has to invest to get equal fitness returns.

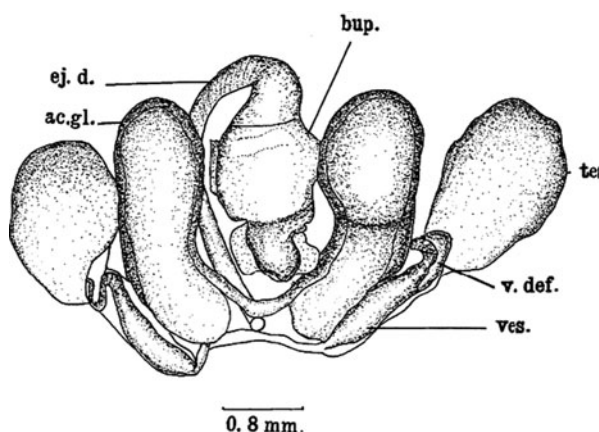
The efficiency of drone production, defined as the chance for a drone to mate seems to vary much throughout the year; however, most of the drones produced in the winter period are chased away from the hives by workers and die soon after their production. In addition, the number of young queens produced was very low. Why did our colonies produce so many drones when they were killed shortly afterwards and when very few virgin queens were available to mate with in wintertime? Unfavourable conditions after the start of drone rearing apparently make production inefficient in winter. It indicates that rearing of drones is the expression of a certain surplus capacity in brood rearing (Velthuis 1990), and immediately responds to resource availability.

An interesting observation on the construction of drone cells was made. Colonies of *A. mellifera* re-use their constructed drone cells many times, whereas colonies of *A. cerana*, at least in this study, only seem to re-use newly constructed cells during the same period of drone production. Old cells are destroyed during the dearth periods. The question is why do these bees destroy empty drone cells when they can re-use them only about 1 month later. During the dearth periods, colonies minimize brood rearing in response to unfavourable conditions. Workers cluster at the centre of the colony. We observed that the comb quickly gets old, black and brittle when it is empty. Workers probably gnaw out unused cells to prevent infestation of wax moth, a common pest in the tropics. Because the drone cells are usually on the outer rims of the comb, they are destroyed before the worker cells. In this study, basic data related to production of sexuals in *A. cerana* colonies have been presented. The data are similar to data from the much better studied Western honeybee, *A. mellifera*, suggesting that the same mechanisms govern rearing of sexuals. Both the species of bees seem to respond quickly to changes in floral resources by adjusting the extent of brood rearing. This affects colony growth, which in turn affects production of sexuals and eventually swarming. Because during large parts of the year, floral resources are often not sufficient to warrant colony growth, seasonal patterns in the production of sexuals appear.

6.2 Reproductive System of Asian Honeybee *Apis cerana*

The anatomy and histology of the male and female reproductive organs of this bee were described by Kapil (1962a, b). Baehrman (1961) and Simpson (1960) described everted and uneverted endophallus of this bee, respectively. Sharma (1960) found repeated matings in two out of nine virgin queens observed by him.

Fig. 6.1 A dorsal view of the internal male reproductive organs of a newly emerged drone, *ac. gl.* accessory gland, *bup.* bulb of the penis, *ej. d.* ejaculatory duct, *tes.* testis, *v. def.* vas deferens, *ves.* vesicula seminalis. (Source: Kapil 1962a)



6.2.1 Male Reproductive System

6.2.1.1 Anatomy and Histology of the Reproductive Organs

The male reproductive organs (Fig. 6.1) consist of all the usual fundamental parts described for *Apis mellifera* (Snodgrass 1925).

The Testes

These are a pair of oval, creamy, voluminous bodies, lying latero-dorsally on either side of the ventriculus in the newly emerged drones. With advance in age, these shrink gradually and ultimately in the old drones these get shifted back from their original position than in the young drones and come to lie at the level of the cephalic end of the accessory glands. In shape in the pupae 4-day before emergence (Bishop 1920) and in the adults (Snodgrass 1925) the testes resemble those of *Apis mellifera*, but their colour does not change from creamy-yellow to greenish yellow in *Apis indica*. Each testis is composed of about 109 tubules. Bordas (1895), Machida (1934), D’Rosario (1942) and Imms (1957) have reported 200–300 testicular tubules in vespids and the honeybee, whereas in other bees and wasps the number mentioned is 1–4. These are ensheathed in a white opaque membrane, which in the early pupal stages is thin and transparent. In the newly emerged drones and pupae, the membrane is covered with branched fatty masses, staining readily with Sudan black. Bordas (1895) mentions that in all the higher Hymenoptera a single testicular capsule covers both the testes; only difference being that its thickness in different families and subfamilies varies considerably. Few genera were held out as exceptions in having independent covering for each testis. Each tubule of the newly emerged drone is 0.89 mm long. The tubules run from the anterior to the postero-lateral end of the testes.

The Testicular Capsule

The epithelial sheath of the wall of the testicular capsule *consists* of two distinct layers as described by Koschevnikov (1891) in *Apis mellifera*, but in detailed histology, the description of the two layers is somewhat different. In *Apis indica*, the outer layer of the sheath is composed of lightly stained cytoplasm with rounded nuclei (0.0159 mm in diameter) in contrast to the inner layer, which is deeply stained and has about 0.0077 mm long, flattened nuclei. The latter are not distinctly spindle-shaped as has been described for *mellifera* (Koschevnikov 1891) and the cytoplasm is also, not fibrous but is coarsely granular. The differences in appearance of the cytoplasm and the nuclei have perhaps occurred because of fixation artefacts appearing in the case of *mellifera* since the structures here, have been found similar in more than one fixative. The outer fatty envelope in *Apis mellifera*, actually corresponds to the fatty masses in *indica*. These by no means are a true layer, for these do not cover the testes on all sides and are mostly localised at their anterior ends.

Unlike the description of Koschevnikov (1891) the tracheae and tracheole in *indica* not only penetrate the outer layer of the testes and have been found to be ramifying among the testicular follicles, but these have also been observed penetrating the follicular wall and reaching deep into the element of the follicles among the spermatids. Their presence among spermatids explains their function as oxygen supply lines to the growing cells and probably for relieving them of gaseous poisons. A similar case was reported by Ruckus (1919) while describing the reproductive organs of *Corpocapsa pomonella* (Lepidoptera) and arrived to a similar conclusion. In a pupa 4 days before emergence, when the pigmentation of the eyes start, the testicular tubules are filled with the spermatozoa, leaving the anterior region mostly empty, where the spermatids of two sizes are often present. The nuclei of the smaller spermatids stain deeply with gentian violet than those of bigger ones.

This means that most maturation stages pass earlier than the 4-day age of the pupae. Bishop (1920) and Zander (1951) have earlier reported that sperm-formation is accomplished in *mellifera* before the individuals reach the imaginal stage. However, they do not particularise the age at which this actually happens. An almost similar instance has been reported in *Camponotus pennsylvanicus* (Forbes 1954) and while comparing the physiological reproductive function of the two animals—*A. mellifera* and *C. pennsylvanicus*—he has not considered the point whether the phenomenon occurs at the same age when the function may be assumed to be, though unlikely, at the same level. In view of this, his approach appears inconsistent, for the animals under examination were different.

In old drones, the tubules are lined with vacuolated cytoplasm, in which oval nuclei are irregularly distributed. The vacuoles are partially filled with light pink granular matter having appearance identical with the secretory granules. The tubules at this stage of the drone's life are completely devoid of spermatozoa or their developmental stages. Because of the foregoing changes, it appears that the testes at a 12-day stage becomes functionless and the spermatozoa are driven into the testicular chamber and vas deferens.

The Testicular Chamber

It is a narrow parochial cavity, situated postero-laterally towards the inner margin of the testes. The tubules open individually into the chamber on its inner wall. The outer wall of the chamber is straight and is opposed to the peritoneal sheath. Its epithelium is synchial and consists of finely granular cytoplasm with oval, centrally placed nuclei. In old drones, the vas deferens extends into the posterior portion of the testis but this condition is not met with in the young drones and pupae. This point nevertheless is not clearly explainable from the above observation as to why in the old testis the anterior end of the vas deferens introverts into the chamber, yet it shall be fairly reasonable to think that it has some sort of relation with the shrinkage of the testes

The Vasa Deferentia, Seminal Vesicles and Accessory Glands

The anatomy and histology of the vas deferens, seminal vesicles and accessory glands do not differ to a marked degree than those of *Apis mellifera* (Bishop 1920). Hence, only certain points of interest have been discussed. The epithelium of the seminal vesicle is made up of glandular cytoplasm projecting inwardly as three longitudinal ridges. The cytoplasm shows divergent affinities for stains—outer half is acidophilic and the inner basophilic—having an accumulation of basophilic granules.

The cells of the accessory gland are glandular, columnar (0.119×0.004 mm in size) with haematoxylinphilic cytoplasm and oval nuclei, as wide as the width of the cells. In old drones, the epithelium at the anterior part is much reduced (0.015 mm broad) while in the newly emerged drones it is nearly 0.17 mm. Bishop (1920) mentions that during advanced stage of the secretory activity, the gland distends so much with the secretion that in its anterior part the epithelium completely disappears. It has been observed that the epithelium gradually gets thinner and is an eighth of a millimeter in breadth, in a profusely secreting gland. It is entirely absent only at the tip. Anteriorly, the musculature is rather weak. The circular and longitudinal muscles are about 0.017 and 0.33 mm thick respectively. In the posterior region, the circular muscles are well developed (about 0.06 mm thick). From the centre of the gland, another muscle layer begins, which proceeds posteriorly and enters into the muscular complex, formed at its base (described by Bishop). Laterally, the inner muscles, which have a criss-cross arrangement in the accessory gland, extend as narrow muscular strands below along the sides of the basal pocket and ejaculatory duct. The circular and external longitudinal muscles run as a rule parallel, but a short distance below the junction of the ejaculatory duct with the basal pocket, the external longitudinal muscles take an inward bend in such a way that the circular muscles become external and the longitudinal muscles internal toward the inner posterior part of the gland. The muscles from the other limb of the gland join at the base in a U-shaped bend thereby forming thickened muscle layers.

The Ejaculatory Duct and its Junction with the Accessory Glands

The ejaculatory duct is a ventrally placed slender tube, originating from the junction of the limbs of the accessory glands.

The Copulatory Organ

Detailed anatomy of the copulatory organ and external genitalia has been described in *Apis mellifera* (Snodgrass 1925; Arnhart 1937). Here it is almost the same, except that the spines either on the plates or on the general tubular parts of the penis are generally directed backward and these suggest that these do not offer resistance and allow the drone to copulate freely. However, the backward movement of the penis is obviously not possible and the arrangement provides a strong grip inside the bursa copulatrix of the queen bee; that is why the drone is unable to separate as soon as the process of copulation is complete. It is perhaps a device analogous with the formation of plug in some Lepidoptera to allow sufficient time to the spermatozoa to get collected into the spermatheca.

The epithelial cells are columnar and lie in continuation with those of the ejaculatory duct. Posteriorly, the cells are, however, smaller in size. The membranous intima extends into the penis bulb, but its width varies in different regions. The ejaculatory duct opens into the bulb of the penis from a small opening, formed by the membrane itself. This portion has been termed as the projected ring-wall in *mellifera* and the opening has been shown to be formed by the projecting wall with unpaired semen directors (Arnhart 1937) and not by the membranous intima. This opening seems to serve as a regulatory aperture for the seminal fluid and since the ejaculatory duct has no muscles, it evidently works with the help of pressure exerted by the body fluid and abdominal muscles.

The presence of secretion in the cavity of the bulb around the inward projected wall, a structure corresponding to the wedge of ants (Forbes 1954), obviously supports the hypothesis that it serves as a second storage of the seminal fluid (first storage is considered as the seminal vesicle by Bishop 1920). The posterior inward projected wall before ejaculation appears to affect control on the forward flow of the seminal fluid. It is pushed down and is formed into a funnel-shaped structure (Arnhart 1937), when the penis is ejected to allow seminal fluid to pass into its tubular part and then down. The penis since is devoid of muscles, it is obvious that the movement of the penial organ is brought about because of pressure of the insect blood and abdominal muscles. Certain studies on behaviour and cytogenetical problems (Deodikar et al. 1959) have minimised gaps in our knowledge on the affinity of two domesticated *Apis* species. It is now increasingly felt that the two species are closely related—a morphometric relation of high altitude *Apis indica* varieties with certain races of *Apis mellifera* has been reported (Kapil 1956). And on account of the presence of all the four species of *Apis* in this and neighbouring countries, Indo-Malayan region has been considered as the original home of *Apis mellifera*, and *Apis indica* as the mother species, which has migrated in course of time to other parts of the world

and has evolved into different races. It is clear that the differences on the number of tubules and shrinkage of the testes with advance in age are actually too minor to be considered as of significance. In most details, the reproductive organs of *Apis indica* and *Apis mellifera* resemble. Some points of interest have been summarised below:

1. The testes are oval, creamy bodies in the newly emerged drones. In old drones, these are reduced, conspicuously yellow structures, much shifted back from their position in the young drones. They are composed of 109 testicular tubules. The shrinkage in the size of the testes with advance in age has been studied.
2. The epithelium at the anterior end of the accessory gland is attenuated in old drones. The course of the muscles in and beyond the junction of the basal pocket and ejaculatory duct has been traced out.
3. The constituting wall at the junction of the basal pocket and the cone of the ejaculatory duct has been described as consisting of reticular cytoplasmic element with a large number of nuclei.
4. The ejaculatory duct opens into the roof of the penis bulb by a small opening formed by the membranous intima. The posterior end of the bulb has an inwardly projected wall with an opening in its centre. This has been interpreted to serve as a control for semen before ejaculation. The penis bulb acts as a second storage.

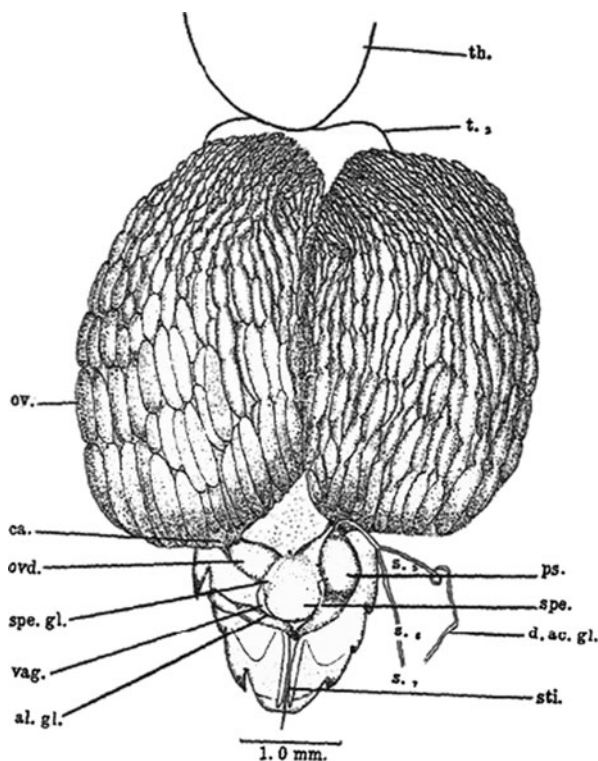
6.3 Female Reproductive System

The female reproductive system consists typically of a pair of ovaries, a pair of lateral oviducts, vagina and the bursal pouches.

6.3.1 The Ovaries

The ovaries of a normal or drone-egg-laying queen bee (Fig. 6.2) are a pair of huge bodies lying ventro-laterally on either side of the alimentary canal. These extend from second up to the middle of the fifth, and from third to the sixth visible abdominal segments in the queen bee and the worker bee, respectively. Each ovary of the queen bee is composed of about 73 polytrophic ovarioles, with narrow curved anterior ends, turned inwardly and attached with one another. In the worker bee, the ovarioles vary from 1–12 in the right and from 1–9 in the left ovaries. In general, the right ovary possesses larger number of ovarioles or the number is equal in both. In *Apis mellifera* queen bee the ovarioles are numerous but their number is inconstant (Imms 1957) and in the worker bees, 13 % possess an average of 4–8 ovarioles (Hess 1942, from a study of 4,000 specimens). In *Apis indica cerana* the ovarioles range from 3–30 and in *Apis mellifera* from 1–26 (Sakagami and Akhira 1958). Kapil (1962c) in his study on 58 egg-laying worker bees found 15.5 and 24 % ovarioles in the right and left ovaries, respectively (which possess four ovarioles). Hess (1942) reported

Fig. 6.2 Dorsal view of the female reproductive organs of the queen bee. *at. gl.* alkaline gland, *ca.* calyx, *d. ac. gl.* duct of the acid gland, *or.* ovary, *m. d.* oviduct, *ps.* poison sac, *spe.* spermatheca, *spe. gl.* spermathecal gland, *sti.* sting, *s₅* fifth sternum, *s₆* sixth sternum, *s₇* seventh sternum, *t₂* second tergum, *th.* thorax, *vag.* vagina. (Source: Kapil 1962a)



maximum ovarioles in the left ovary. Sakagami and Akhira (1958) reported in *Apis indica eerana* maximum number of ovarioles as 30 in the right ovary and in *Apis mellifera*, 26 in the left ovary. Here the highest number is 12 in the right ovary.

The ovaries of the freshly emerged queen bee extend from second up to the fourth abdominal segment. Being comparatively smaller in size, these do not crowd the abdomen, although because of air sacs surrounding them, they appear to occupy the whole of the abdomen. The ovaries are covered with fatty cells, which are also present between the ovarioles. The fatty cells occasionally are densely granulated and their structure resembles those of over-wintered early April bees (Snodgrass 1925). The ovaries of the drone-egg-laying queen bee do not differ externally and histologically except that they have at times been observed to possess black pigmented fatty cells. The ovaries of the normal worker bees and of those, which have stopped, functioning due to emergence or introduction of the queen bee, are much reduced bodies, hardly traceable among the surrounding tracheae. On the other hand, the functional ovaries become enormously enlarged and reach as far as the anterior end of the abdomen. The histology of the ovary and the development of the oogonia have been described in *Apis mellifera* (Snodgrass 1925) and in *Apis indica* there is no difference. The point of interest here lies in the manner in which the oocyte in its latter stages establish connexion with the nutritive chamber to draw nourishment for growth when the

polytrophic character has already been made up. The process to make connexion for nourishment as it appears from the observations, can occur by two probable ways. First, that the wall is broken by an internal pressure exerted by the protoplasm of the oocyte or secondly that there is some sort of dissolving substance released by the protoplasm of the oocyte or the nurse cells, which help dissolve the intervening wall. Since the protrusion of the protoplasm from the oocyte initially plays a dominant part and the disappearance of the wall has been observed as a gradual process, before giving away completely, it lends support to the idea that the process occurs by dissolving a secretion of the nurse cells, which have been known to secrete (Schneider 1917). The protrusion of the protoplasm perhaps provides a stimulus. The wall of the ovariole in the newly emerged queen bee is considerably thick. Each ovariole has throughout its length, oogonia and some transitory stages leading to the development of the oocyte. The late or mature stages are absent and differentiation into the nutritive chamber and mature oocyte has not yet taken place. The ovary hence does not present tile polytrophic character, which probably appears after the mating has accomplished. In the old ovaries, the nurse cells on being apparently exhausted, have been observed to degenerate showing at the same time numerous black granules of variable shape and size.

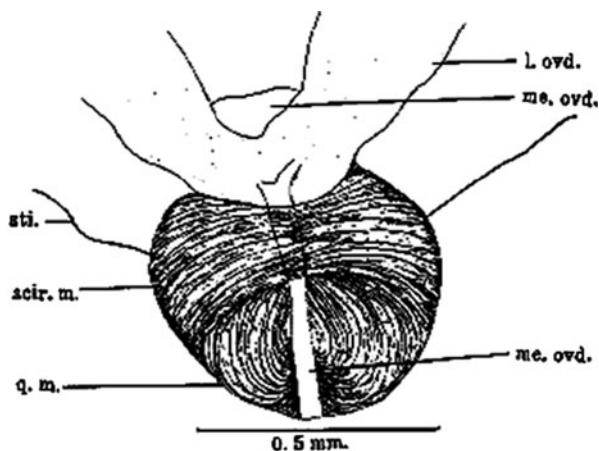
6.3.2 *The Calyx*

At the caudal end of each ovary of the queen bee, there is an irregular, thickened portion, which corresponds to the calyx. Histologically, it consists of a layer of ectoplasm having oval nuclei. Empty spaces are often observed in the cytoplasm, some of which have been found filled with basophilic granules. The calyx opens into the lateral oviduct. The ovarian follicular cells, from which the oocyte has already passed out, have been observed in it. These cells stain deeply with haematoxylin and have been termed as corpus luteum (Wigglesworth 1953). Their remnants are also found in some cases. The calyx in the workers' female reproductive system is a thick-walled, nearly 0.169 mm long structure between the ovary and the lateral oviduct. Anteriorly, it is somewhat solid having an opening lined with an irregular intima. This appears to afford considerable distension, allowing the mature oocyte to pass down into the oviduct. The cytoplasm of the syncytial epithelium stains deeply around the axis than the periphery and has a number of nuclei, rich in chromatin granules. Bordering the epithelium are about 0.012 mm thick circular muscles.

6.3.3 *Oviducts*

The paired lateral oviducts unite with the median oviduct by a small opening nearly 0.39 mm below their anterior ends. The spine-like structures in *Apis mellifera* (Mori-son 1928) and ridges or folds (Laidlaw 1944) seem to be identical structures and appear occasionally as spurious fixation products. The syncytial epithelium of the

Fig. 6.3 Dorsal view of the median oviduct showing arrangement of the major muscles, *l. ovd.* lateral oviduct, *me. ovd.* median oviduct, *q. m.* quadrant muscle, *scir. m.* semi-circular muscle. (Source: Kapil 1962a)



ducts is surrounded on outside by the longitudinal muscles (Fig. 6.3). In the median oviduct the muscles have attained a specialised circular contour and have been termed as semicircular and quadrant muscles (Laidlaw 1944). Besides the first and second tergoventral muscles, another band of muscle fibres, the median internal ventral muscle, passes dorsally above the vagina and the bursal pouches, and is directed antero-ventrally to attach itself on the last sternum. The muscle consists of a pair of outer fibres and a deep median fibre. Because it stretches over the vagina and the bursal pouches anteriorly and up to the base of the sting posteriorly, it appears to work antero-posteriorly, helping the queen bee to push the eggs forward (Fig. 6.4).

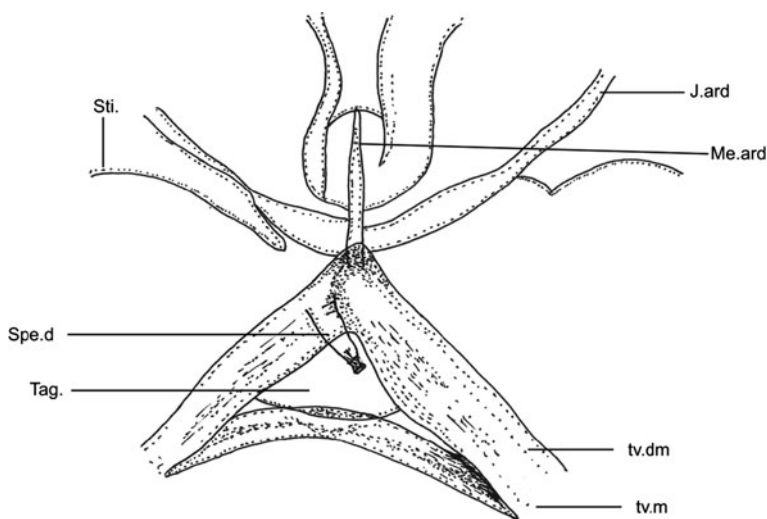


Fig. 6.4 A diagram showing a dorsal view of the median oviduct the muscles and opening of the spermathecal duct into the vagina, *spe. d.* spermathecal duct, *tv. m.* tergo-ventral muscles. (Source: Kapil 1962a)

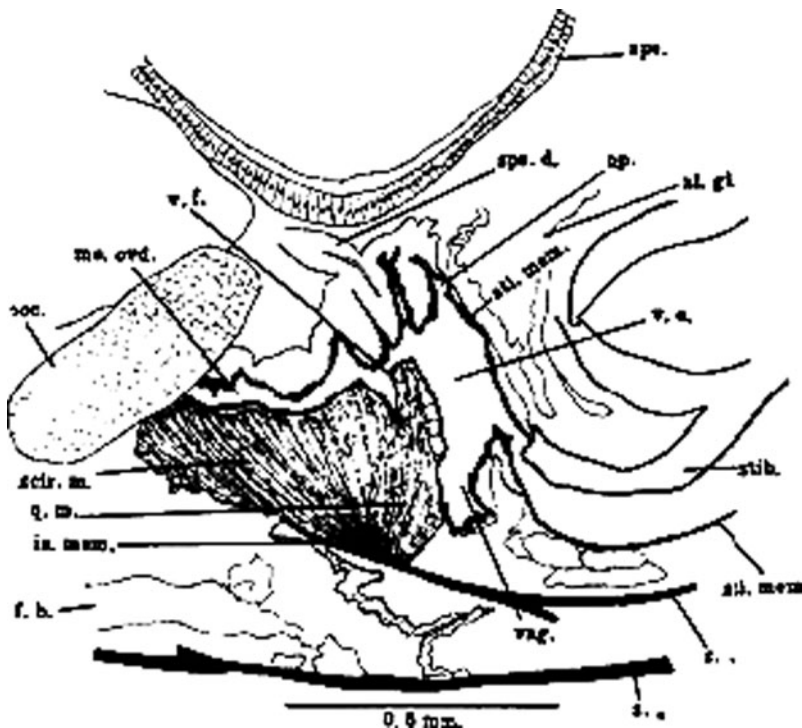


Fig. 6.5 L. V. S. of the abdomen of the queen bee showing *lateral* view of the reproductive tract (reconstructed from hand sections), *b. p.* bursal pouch, *b.* fat body, *is. meb.* intersegmental membrane, *sti. meb.* sting membrane, *v.f.* valve-fold, *v. o.* vaginal opening. (Source: Kapil 1962a)

The flattened and other muscle fibres (Laidlaw 1944) in fact are constituent parts of the quadrant muscle fibres. The lateral oviducts comprising the vagina in the worker bee resemble histologically the oviduct of the queen bee. It has been observed that the eggs block the lateral oviducts of the worker bees and these have often been found in them nearly at the same level. From this, it appears very likely that both the ovaries function simultaneously and naturally, the eggs descend approximately at the same time. In addition, it has been observed that the reproductive organs of the worker bee have no developed system of muscles of their own, which may place the worker bee in a position to retain the eggs for a sufficient period, enabling her to lay them in an organised manner. The result of this deficiency is that she lays more than one egg in a comb cell.

6.3.4 The Valve-Fold

It is a ridge-like two-walled structure (Fig. 6.5). The anterior wall begins form the base of the median oviduct anti rises upward to meet the posterior wall. The valve is inclined anteriorly. Its inclination appears to have a significant functional relation

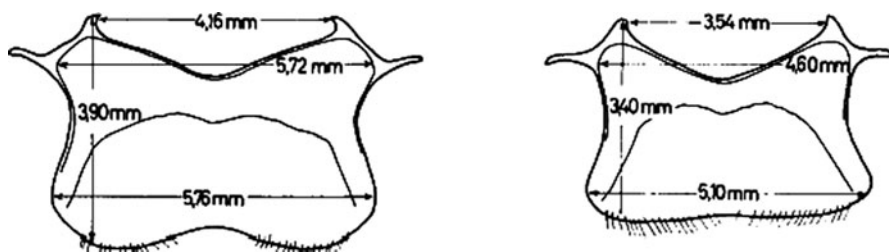


Fig. 6.6 Third sternal sclerite of queen: *A. mellifera* (left), *A. cerana* (right)

with the opening of the spermathecal duct, which is likewise somewhat anteriorly located. It means explicitly that the valve-fold controls the opening and flow of the sperm into it for storage and on their return journey to fertilise the eggs. Controlling function of the opening by the valve-fold and prevention of the sperm from upward suction when the sperm pump works, was first pointed out by Bresslau (1906) but Adam (1913) suggested that the fold holds the micropylar end of the eggs against the opening of the spermathecal duct to ensure fertilisation.

From the attachment of the muscles on the anterior and posterior walls, it is obvious that the walls are movable antero-posteriorly, and can be drawn to make way for the eggs when they move down. With the help of the semicircular and quadrant muscles, the wall of the median oviduct contracts to copulatrix. It would be expected that the valve-fold perhaps, lies anterior to the spermathecal opening when the mating takes place, permitting the sperm to be sucked into the spermatheca via the ducts and not into the median oviduct. On the other hand, when the egg descends the valve acquires just the reverse position; that means it rests posterior to the opening and holds the micropylar end of the egg close to the opening of the spermathecal duct for fertilisation before it is allowed to pass on.

6.3.5 The Spermatheca and the Spermathecal Gland

The structure and histology of the spermatheca and its duct have been fully described in *Apis mellifera* (Bresslau 1906). In *Apis indica* the details are the same (Fig. 6.6). The histological structure of the spermathecal gland is of interest here. It consists of glandular columnar epithelial cells, built on a distinct basement membrane (Figs. 6.7 and 6.8). The nuclei in it are of two types. Those lying at the base are bigger, somewhat round (about 0.014 mm in diameter) and have four or five chromatin granules. The smaller or endothelial nuclei are 0.004 mm in diameter and have a chromatin granule. The lumen of the gland is lined by a thick intima. Each cell has a ductile, terminating in the lumen of the gland. The nerve innervating the gland is attached with the basement membrane by the connective tissue. The endothelial nuclei have earlier been reported in the colleterial glands of Orthoptera (Fenard 1896) and in the mandibular glands of *Apis indica* (Kapil 1958). Kratky (1931) called them

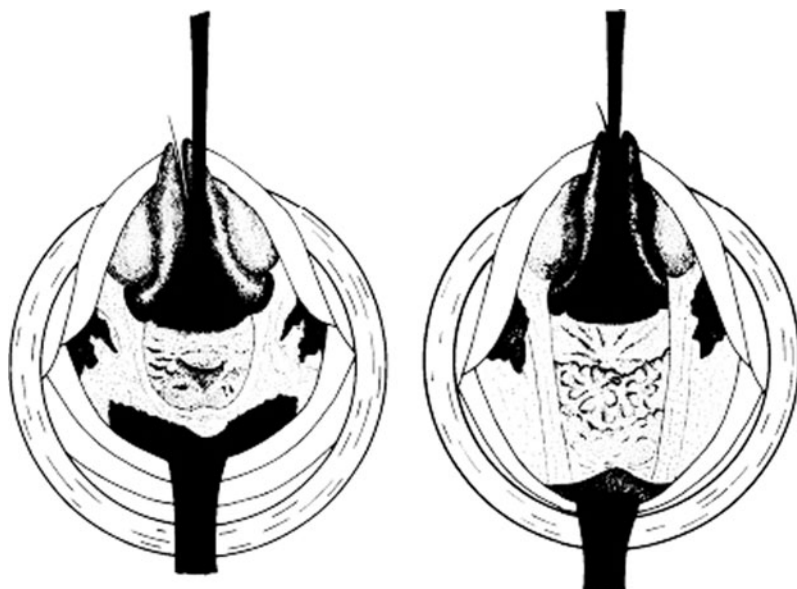


Fig. 6.7 Sting chamber of queen (opened by hooks for insemination): *A. cerana* (left), *A. mellifera* (right)

nucleoli in the mandibular glands of *Apis mellifera*. They appear to play an active role in the secretory process and have been seen in quite a large number in the lumen (Kapil 1958). Here they appear to be in the resting stage as the gland was studied for the newly emerged queen bee. The spermatheca in the laying worker is a rudimentary structure. It is wider proximally than distally and opens nearly in the centre of the vagina. The rudimentary bursa copulatrix, the spermatheca and the spermathecal gland have been reported in the reproductive system of the laying worker of *Apis mellifera* (Hambleton 1928) but Hess (1942) reported only the spermatheca.

6.4 Evolutionary Tendencies and Affinities of *Apis indica* with *Apis mellifera*

In deriving a relation between the two domesticated species of *Apis indica* and *mellifera* Kapil (1956) in a comparative study of the morphometrical characters of the varieties of *Apis indica* has clearly shown that the hill variety is relatively bigger than the plains variety. The tongue length for the hill variety has been reported as 5.5184 mm, in contrast to the smallest tongue length of the Italian bee in USA, which is 5.896 mm (Alpatov 1929). The other records on the tongue length of *A. indica* are still less. This means that the bees have travelled north and have adapted in the course of time, developing longer tongues probably to reach the deep-seated

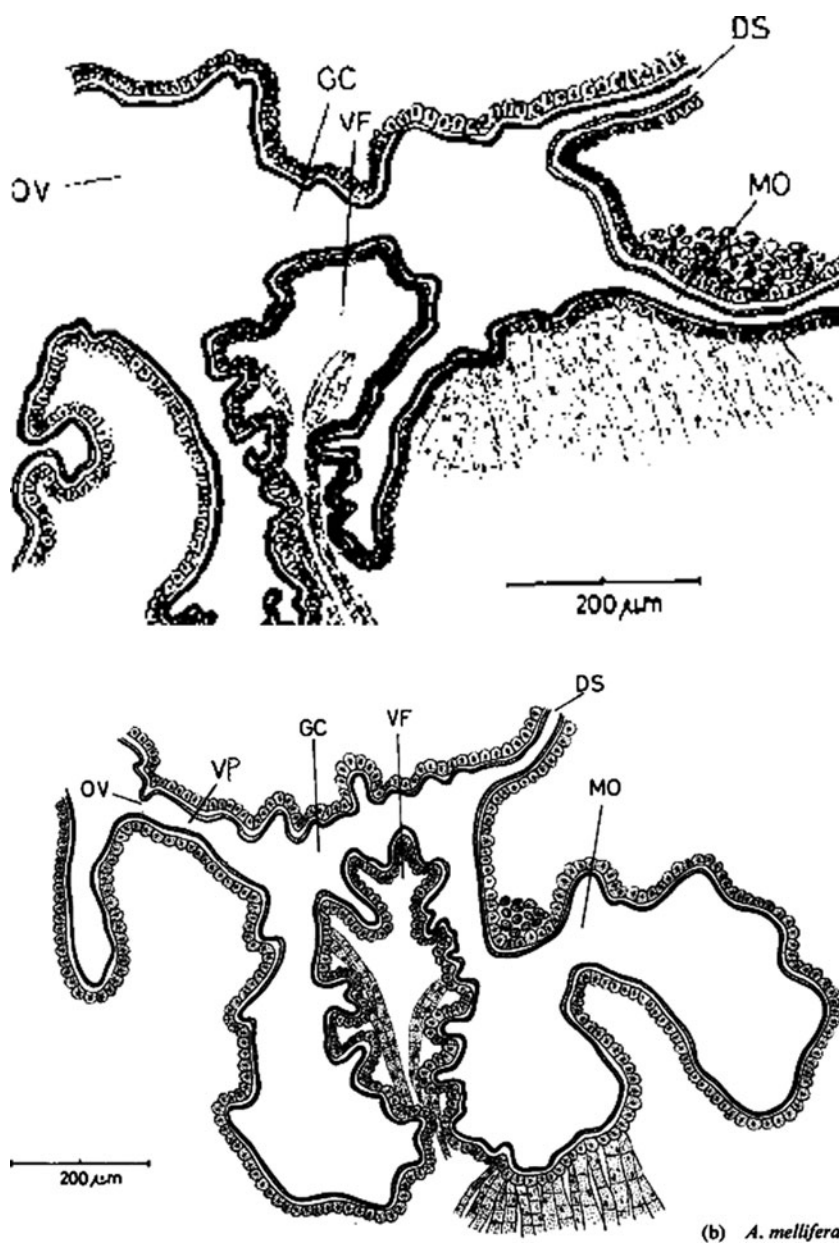


Fig. 6.8 Vagina and oviduct, mean sagittal section. *DS* spermathecal duct, *OV* vaginal orifice, *GC* genital chamber, *VF* valve-fold, *MO* median oviduct, *VP* vaginal passage

nectaries of flowers in those regions. Since there is a marked increasing trend in size, in all probability and may not be quite precisely, that if most intermediate forms are worked out, a still closer relation can be established. The evidence is furthered by a report on the presence in southern India of the smallest sized bee of *A. indica* (Ratnam 1939), which exists along with other primitive forms there and retains a primitive behaviour shimmering and absconding that has completely been forsaken by *A. mellifera* (Butler 1954). Hence, it appears quite logical to suppose that the original home of the living species of *Apis* was in southern hemisphere, possibly in the Indian sub-continent or in Indo-Malayan region where other primitive bees with species of *Apis*—*florea*, *dorsata*, *indica* and *mellifera*—are yet found. The wing length of the hill variety of *A. indica* is close to the Sicula (Kapil 1956). In most details the male reproductive organs of *A. indica* (Kapil 1962) resemble those of *A. mellifera*, except for differences in the number of testicular tubules, which are about 109 in the former and 200–300 in the latter (Imms 1957a) and shrinkage of testes with advance in age which is 1:3.33 (in *A. mellifera* (Bishop 1920) and 1:2.85 (in *A. indica*, Kapil 1962a, b, c). The female reproductive organs in general details are similar in the two species but for the number of the ovarioles of the queen bee which are an average of 73 in *A. indica* and in *A. mellifera* these have been reported as numerous but inconstant (Imms 1957). The ovarioles of the worker bee range from 1–12 in *A. indica*, 3–30 and 1–26 in *A. indica cerana* and *A. mellifera* (Sakagami and Akhira 1958). Hess (1942) reported an average of 4–8 ovarioles in *A. mellifera*. Deodikar et al. (1959) have shown a clear similarity in the number of chromosomes in both the species. The number reported in male is 16 and in female 32. The foregoing discussion obviously gives rise to three points: (i) that there are no differences of significance in the structural details; (ii) that the morphometrical characters of the honeybees have higher values in the north than in the south and (iii) that the increase in size of the organs and in the number of the testicular tubules and ovarioles are adaptations for survival of the tribe. Although there are no definite gradational data available to show linkage in the increase in number and size of the organs, the positive tendencies of lineage are far more suggestive.

The dependence of the worker bees more and more upon the flowers which became for them main source of food, resulted in lengthening of their tongues (Butler 1954) and wings (Carlisle 1955) in the temperate and sub-arctic regions where the conditions were assumed as much harsher than the tropics (Butler 1954). The increasing trend in size in both the temperate (Carlisle 1955) and tropics (Kapil 1956) extends from the south to the north. Evidently, with the specialisation of the external organs, it may be expected that the internal organs likewise pace ahead hand in hand with the external ones. Result being that for survival and maintenance of the social set-up of the colony the queen bee in the temperate region must lay larger number of eggs (Philips 1945; Park 1954), compared with one in the tropics (Kapil 1957), and this requires an increase in the number of its ovarioles. Moreover, the queen bee since normally mates once or twice in life, barring exceptional cases, and lives comparatively longer than its counterpart in the tropics, naturally it must receive fairly large number of the spermatozoa so that the required number of eggs are fertilised to produce a force of worker bees to keep up the colony in all its activities. The latter

function has therefore necessitated the development of larger number of the testicular tubules in *A. mellifera* to conform to the needs of colony building. The long-lived and potential egg-laying queen bee of *A. mellifera* has been probably, on the other hand, a factor to counteract the urge of the worker bees to develop larger number of the ovarioles in *A. mellifera*. In addition, it seems quite reasonable to think that those conditions have, a conspicuously low level, decreased instability of the colonial life, as opposed to the *indica*-group by suppressing the absconding. However, it would be premature to assess a strict relation on some of the latter observations, owing to little data available in this respect, particularly in the *indica*-group. To sum up the above discussion it may be noted that it lends support to the popular contention of many workers that it was *A. indica*, which migrated in different directions, especially towards north, and has specialised to become what we know today as *A. mellifera*. In addition, while maintaining many structural similarities, it has eminently transmuted itself for some under the pressure of environment

6.5 Mating System

It has been well established from the early days of science (von Berlepsch 1873) that neither a worker bee nor a drone or a queen can exist alone without the social context of the colony thereby favouring concept of “super-organism” (Gerstung 1910; Moritz and Southwick 1992; Moritz and Fuchs 1998; Hölldobler and Wilson 2009). This could be compared to the body parts of a higher organism—each individual member of a highly developed insect society has lost the ability to survive without its colony. Nevertheless, the individual honeybee and especially queens and drones are still subject to natural selection. Individual selection, however, is tightly interwoven with a complex selection at colony level.

6.6 Social Structure

In general, honeybee colonies are monogynous, having only one queen which lives together with thousands of workers. In terms of reproduction, a successful establishment of a daughter queen is inevitably linked to investment in large numbers of worker bees and only colonies, which are big enough for swarming (colony fission) and can invest in daughter queens. In comparison, drone production does not involve anything more than the rearing and maintenance of individual drones.

6.7 Daily Mating Flight

Apis drones meet at drone congregation areas in large numbers and these drone congregations start independently of the queens (Ruttner and Ruttner 1965; Koeniger and Koeniger 2000). Drones fly their waiting loops at the drone congregation areas long before the first queen starts out for a mating flight. The landmark, which

guides the drones, and later the queen, to the drone congregation areas, seems to be prominent tree canopies in Asian honeybees. The landmarks for *A. mellifera* drone congregation areas are still unknown. The number of drones commuting daily between a drone congregation area ranges from several hundreds to about 16,000. Further, a large number of colonies in the surrounding area also contribute to the drone population of drone congregation areas. In conclusion, the *Apis* queen meets a large number of drones from many colonies at drone congregation areas. Thus, the negative consequences of inbreeding in honeybees have resulted in a most panmictic mating behaviour.

Mating season and reproductive swarming in honeybees depend on the availability of ample pollen and nectar. Honeybee swarms are very vulnerable to food scarcity. Therefore, the mating season of honeybees generally depends on flowering cycles and under sympatric conditions, honeybee species produce drones and queens simultaneously. The encounter of alien sex partners (drones and queens of different *Apis* species) is avoided by well-separated species-specific, daily mating periods.

6.8 Queen Polyandry

In honeybee colonies, queen polyandry is a common phenomenon and number of effective paternities ranges from about 12–18. However, there are exceptions in each subgenus. In the subgenus *Apis*, *Apis nigrocincta* queens have a significantly higher mating frequency. In *Megapis*, *Apis dorsata* colonies also have a higher paternity number, and in *Micrapis*, *Apis florea* colonies have a lower paternity number. A change of paternity numbers has evolved three times independently, and the balance between the costs of additional mating and the benefits resulting from a high number of paternities on the colony level seems to be subject to a high selective pressure. In conclusion, the asymmetry of mating behaviour between the sexes—the ultimate monogamous drone and the extremely polyandrous queen—has reached an extremely high level in the genus *Apis*.

6.9 Competition Among Drones

The extremely male-biased sex ratio has resulted in a high rate of competition among drones. The drone's sensory input is optimised towards fast queen recognition. The huge compound eyes cover the whole upper part of the head. The number of ommatidia in drones is much higher than in workers or queens and the spatial resolution is well developed. These functional adaptations are more or less uniform throughout the genus *Apis*. Adaptations of queens to flight speed and detection of pheromones are, in comparison to their male counterparts, less expressed. Thus, selective pressure caused by the male-biased sex ratio has been focussed on the drones, while queens remain more or less unchanged (besides the unquestionable adaptations to high egg production).

Fig. 6.9 Partly everted endophallus of *Apis cerana indica* drone. *BC* bursal cornua, *CE* cervix, *UC* upper cornua

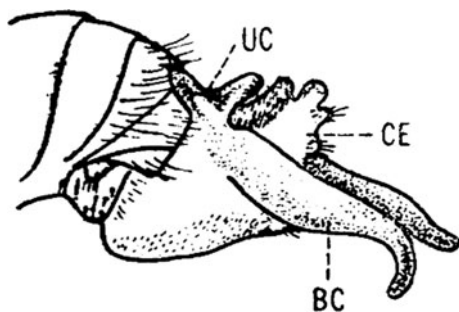
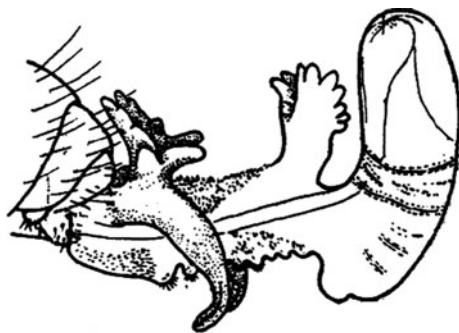


Fig. 6.10 Stage of eversion of the endophallus in which the semen should be collected for artificial insemination. *SE* semen



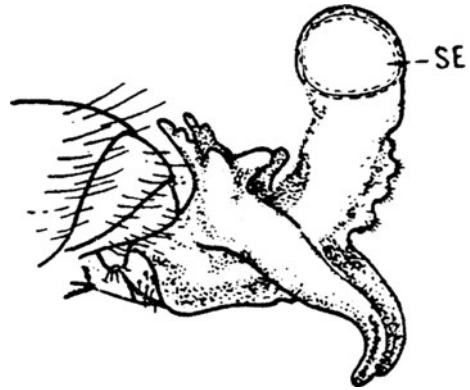
6.9.1 Monogamy in Drones

The extreme drone-biased sex ratio in the genus *Apis* significantly reduces a drone's chance to successfully mate. The probability to mate even a second time is very close to zero. So natural selection has favoured drones, which performed a “total investment”, whenever they got a rare chance to meet a young queen. Common among all *Apis* species is the general anatomy of the male genitalia, which consist mainly of a densely folded membrane packed inside the drone's abdomen. Therefore, the organ—specific to the genus *Apis*—has got its scientific name: endophallus (internal phallus). In the course of copulation, the organ is inflated by the drone's haemolymph, and the eversion of this huge endophallus inevitably causes the drone's loss of motility and finally the drone's death. In this regard, the *Apis* drone is a “one-way shuttle designed for sperm transfer”.

6.10 Spermatozoa Transfer

Though the genital tract of the *Apis* queen is well equipped to initiate the ejaculation and the drone's separation, it generally corresponds closely to the female anatomy of Apoidea and other Hymenoptera (Snodgrass 1956). Apparently, anatomical adaptations to typical copulation in *Apis* (during flight) are more conspicuous in drones than in queens (Figs. 6.9, 6.10 and 6.11). Drones of the subgenera *Apis* and *Megapis*

Fig. 6.11 Completely everted endophallus of *Apis cerana indica* drone



deposit their spermatozoa in the oviducts of the queen. In the following hours, the spermatozoa are moved backward from the oviducts to the vagina from where more than 90 % of the received sperm masses are expelled. During this process, spermatozoa of many drones pass the orifice of the sperm duct (ductus spermaticus) and eventually enter the spermatheca. Thus, the spermatheca is loaded with spermatozoa of many drones, which results in an increase of genetic variability among the workers of the colony. In *Micrapis*, the tip of the drone's endophallus is placed directly at the orifice of the sperm duct. The spermatozoa can move directly into the spermatheca. Drones have fewer sperm than a spermatheca can hold and each drone can deposit about half of its sperm load. Thus also in *Micrapis*, the queen's spermatheca contains spermatozoa of several drones.

6.11 Mating Sign

Drones (*A. mellifera*) remove the mating sign by pulling it out with a special hairy field of the endophallus (Koeniger 1986). This is present in drones of all *Apis* species. The idea was put forward that the mating sign might facilitate queen recognition by successive drones. Thus, after being successful in the competition with fellow drones, a drone seems to cooperate "post-mortem" with his successors by marking the queen with a conspicuous mating sign in order to reduce the mating flight time.

6.12 Rearing of Drones and Queens

6.12.1 Drone Rearing

In *Apis*, drones are generally reared from unfertilised eggs. Because drone rearing is costly and drones do not contribute to the economy of the honeybee colony, the

regulation of drone production is a major focal point of natural selection and the main prerequisites for this regulation are different sizes of the comb cell. In dwarf honeybees (subgenus *Micrapis*) and in the cavity-dwelling honeybees (subgenus *Apis*), there are two distinct cell sizes, worker bees are reared in smaller comb cells and drones in the larger comb cells.

Combs in *Apis* are a static and a more or less permanent element. Once the comb is built, the relation of worker cells and drone cells is fixed and cannot be changed unless new combs are built. In the subgenera *Apis* and in *Micrapis*, drone and worker brood cells were counted. In *A. mellifera*, comparison of drone and worker cells in the reproductive season revealed that on average, 5,100 are drone brood and 31,000 worker brood cells, which correspond to 14 % of drone brood of the total brood area (Weiss 1962) while Allen (1965) measured about 16 %. Considering the total area of combs including cells with honey, drone cell area comprises about 10 % in average. In *Apis cerana* and *Apis koschevnikovi*, it is about 11 % (Punchihewa 1994).

Besides brood rearing, comb cells serve many purposes in the subgenera *Apis* and at times when no drones are reared, drone cells can be filled with honey and serve for food storage. In this way, worker bees have the possibility to effectively use the comb and either filling them with honey or emptying them for drone brood production; the drone cells are adjusted to the seasonal requirements of the colony.

In *Micrapis*, the situation is different. In general, at the end of the swarming season, colonies of *Apis andreniformis* and *A. florea* abscond and leave the old comb. The swarms start building a new comb, and during the initial period of colony growth, the bees will build worker cells only and continually extend the comb down and sideward. At the beginning of the next flowering season, drone cells are added at the lower edge of the comb and the downward extension of the comb consists mainly of drone cells. The number is adjusted to the strength of the colony. The queen will lay eggs, and a batch of drones is reared, and with a delay of 10–20 days, queen cells are also built, and with the sealing of the first queen cells the first swarm with the mother queen leaves the comb. From this moment, no further eggs are available and after the last brood has emerged, the last daughter queen with all remaining workers and drones leaves the old comb. The number of worker cells of deserted combs in *A. andreniformis* colonies ($n = 10$) averaged 4,900 and 5,500 in *A. florea* ($n = 6$) and that of drone cells 390 and 570, respectively. Accordingly, in *A. andreniformis* 7.4 % of the brood is drone brood, and in *A. florea* it is 9.4 %. In *Micrapis*, honey is stored exclusively in the top portion of the comb and in contrast to cavity-dwelling species, which also use drone cells for storage.

The queen practises a further regulation of drone production. Only during a limited period will queens oviposit in the larger drone cells. To perceive the size of the cell, the queen carries out an inspection of the comb cell before oviposition. The head together with the front legs are introduced into the comb cell, and during this cell inspection, the queen seems to measure the size of the cell (Koeniger 1970). In cases of large drone cells, the queen blocks Bresslau's sperm pump, so that no sperm is added to the egg during its passage through the oviduct and this unfertilised egg will eventually develop into a drone. In *A. cerana* and in *A. koschevnikovi*, we observed the queen's cell inspection regularly and the behavioural pattern is nearly identical to the

cell inspection of an *A. mellifera* queen. Therefore, we conclude that the recognition of drone cells might be similar in *A. Koschevnikovi* and *A. cerana*. Observations on queens of *A. nuluensis* and *A. nigrocinta* are not yet available. The same holds true for *A. andreniformis* and *A. florea*.

The relationship between drone body size and worker body size differs among Asian honeybee species. In the subgenus *Micrapis*, drones are much larger than workers (in *A. andreniformis* about five to one), in *Megapis* drones are of similar size (about 1.2–1) and the subgenus *Apis* holds a medium position.

A last but very important point of regulation of drone production is cannibalism of eggs and young larvae by worker bees. There is also sound experimental evidence available for *A. cerana* (Nakamura 1995), but there is no doubt that cannibalism of drone brood plays an important role in all species of honeybees. Drones are produced and maintained seasonally, only during the time when colonies can support them and when virgin queens are potentially available. The season matches the time of reproductive swarms and is connected in all species to the blooming season. The peak of drone rearing precedes the rearing of queens. In *A. cerana*, appearance of drone brood is described as a “timely indication for the start of the reproductive season” (Punchihewa 1994). Drones need from 22.5 to 24 days to develop, 2 or 3 days longer than worker bees and about 8 days longer than queens do. This development seems to be independent of the imago’s weight.

6.13 Queen Rearing

Queens in honeybees are not genetically determined. Young female larvae in an early stage are bipotent and can develop into either worker bees or queen bees. The route of development depends on cell type and nutrition (Winston 1987). In the case of queen loss, colonies feed young female larvae in worker cells with royal jelly. They reconstruct and change a worker cell into an emergency queen cell from which fully functional young queens will emerge. Emergency queen cells are known from *A. andreniformis*, *A. florea*, *A. cerana*, *A. koschevnikovi* and *A. mellifera*. The rearing of emergency queens seems to be a mandatory response to queen loss in the subgenus *Apis*. In *Micrapis*, however, queen loss often results in the dispersal of the colony, most often in *A. andreniformis* (Koeniger et al. 2000; Oldroyd and Wongsiri 2006).

Royal jelly as the agent of queen determination does not seem to be identical but is similar across species borders of closely related species. Young larvae of *A. cerana* grafted in colonies of *A. koschevnikovi* were successfully raised to imagines (Koeniger et al. 1996c). Although early experiments to rear *A. cerana* queens in *A. mellifera* colonies failed (Ruttner 1988), the practical difficulties to do this have now been overcome and these two species can produce queens heterospecifically.

For queen rearing, workers build special cone-shaped queen cells at the lower edge of the comb so that queen cells are vertically orientated with the opening below. Thus, they contrast with the other brood cells of the comb, with a more or less horizontal longitudinal axis. The queen cells are rather uniform within honeybees. Only the

size of queen cells differs according to the size of the queens: dwarf bees (*Micrapis*) have smaller queen cells than the cavity-nesting species. Queen cells of *A. dorsata* and *A. laboriosa* have the largest diameter. The number of queen cells per colony depends apparently on colony strength and season but is similar among the honeybee species. They range from 6 to 10 in the dwarf and giant honeybees and from 7 to 15 in the cavity-nesting species (only few data are known for the Asian cavity-dwelling species). In general, several queen cells are constructed almost simultaneously and upon completion, the queen lays eggs in all of them. With the rapid growth of the queen larva, the cell walls are extended downwards. The queen cells are sealed between 7 and 9 days after the larvae hatch from eggs. That seems to be the signal for the old queen to leave the nest together with a fair amount of workers. The developmental time of the three castes is rather similar among the species studied so far, in spite of the large size differences among the species. Drones need the longest period and queens develop fastest; workers are in a medium position of roughly 20 days. This seems to be a principal character for the genus *Apis*.

After the prime swarm with the mother queen has left, the development of the daughter queens ends within a short period. In the cavity-dwelling species in *A. mellifera* to fully develop (Klenk et al. 2004). *A. mellifera* queens begin mating flights only at the age of 5–6 days. Sexual maturation in the dwarf honeybees is faster as queens begin mating flights already at 3 days old (*A. andreniformis*, Koeniger et al. 2000; *A. florea*, Phiancharoen unpublished observation). Queens may leave with an after-swarm before sexual maturation is completed. Drones also undergo a period of sexual maturation. Young drones are found in the brood rearing area where they are fed by the worker bees. In contrast to the larval developmental time, sexual maturation seems quite different among the *Apis* species. Sexual maturation in drones can be best measured by the migration of spermatozoa from the testes to an enlarged part of the vas deferens, the vesiculae seminales, where they are stored for the lifetime of a drone. As in *A. mellifera*, drones of the Asian species do not produce additional sperm after emerging from the brood cell and sperm number is therefore fixed. After emergence, the vesiculae seminales are empty (unpublished observation). Within a few days spermatozoa are transferred continually from the testes to the vesiculae seminales. In *A. dorsata*, the process needs about 12 days, the main migration occurs from the third to the eighth day (Tan et al. 1996). Data on the duration of sperm transfer to the vesiculae seminales are still missing for other species but may be faster. For example, the mean age for the first mating flights was 5.6 ± 1.3 days in *A. florea* drones, while it was 11.1 ± 4.8 days in *A. mellifera* (USA) (Rueppell et al. 2005) and 12 days in Europe (Drescher 1969).

In addition, during sexual maturation, several accessory glands produce the substances which later form a mating sign. The cornua of endophalli of freshly emerged drones are whitish in *A. dorsata*, *A. andreniformis*, *A. florea*, *A. cerana*, *A. koschevnikovi* and *A. mellifera* and become orange only after the cornual gland starts secretion within the next few days (personal observation). In addition, the bulbous glands are active, the secretion forms a ring-like structure in mature *A. mellifera*,

A. cerana, *A. koschevnikovi* and *A. dorsata* drones. *A. mellifera* drones have, in addition, chitin plates, while in *A. florea* the secretory vesicles remain scattered within the cuticle (Moors 2010).

6.14 Sex Ratio

Complete data on the ratio of adult queens, drones and worker bees in the Asian *Apis* species are still lacking. Therefore, we counted drone and queen cells of abandoned combs of the dwarf and giant honeybee species. *A. andreniformis* colonies ($n = 10$) constructed an average of 390 drone and 6 queen cells. *A. florea* ($n = 6$) drone cells averaged at 570 and 10 queen cells. Assuming that only one set of drones and queens are produced per season, the sex ratio of queens and drones is only about 1–60.

In *A. andreniformis*, only two to five queen cells had emerged (recognised by a typical cell opening which was cut by an emerging queen), and in *A. florea*, three to four cells had emerged while the other cells showed signs of destruction. Observations of four colonies revealed that from each colony only two virgin queens were able to start a new colony. These results would change the bias towards the drones by nearly five times. In three cases, the combs were abandoned within 2 weeks; one queen produced one set of brood before leaving (Phiancharoen unpublished observation). It seems that more queens are always reared than in the end are provided with a swarm.

At the edge of six empty *A. dorsata* combs, we found an average of six queen cups in Malaysia, Borneo and 9.7 in Vietnam (Tan 2007). As mentioned above, we calculated about 3,000 drone cells. Thus, the sex ratio would be roughly 1/500 in Borneo and 1/300 in Vietnam, which is in a similar range as *A. mellifera*. For the hive-nesting Asian species, there are only data for one *A. koschevnikovi* and two *A. cerana* colonies (unpublished observation). In these cases, the sex ratio is about or even below 1/100.

These data demonstrate that the sex ratio differs significantly from 1:1 and that the high sex bias of queen and drone brood cells towards males is a general characteristic in the genus *Apis*. This bias in sex ratio can be partly explained by the different costs between queen and drone production. The costs of rearing queens and drones and maintaining them until mating are only slightly different. After mating, the drones die but each queen founding a new colony requires thousands of worker bees.

This leads to the question of whether it is more profitable for spreading the colonies genes by investing in cheap drones than in expensive queens. Evolution surely has resulted in a balance between these two targets but data are still not available. The ratio differs among the *Apis* species from about 1/60 to 1/500. The significance of the “conflict over sex ratio” (Trivers and Hare 1976; Crozier and Pamilo 1996) in honeybees is difficult to assess. Because of the high polyandry of queens and the different proportions of patriline present in the colony, different interests in the sex ratio by worker bees and queens seem to be of minor importance since natural selection in the genus *Apis* seems to operate mainly on colony level (Holldobler and Wilson 2009).

6.15 Mating Flight Periods

6.15.1 Mating Season

Mating season in honeybees and reproductive swarming depend on the availability of ample pollen and nectar. At the beginning, large numbers of workers must be reared. Colonies must reach a high population of workers before swarms are issued. Swarms need immediate access to nectar for comb building and pollen for brood rearing, because they have no other reserves than the content of their honey crops. Whenever bad environmental conditions over an extended period limit foraging success, the survival of a honeybee swarm is at risk. Honeybee swarms are very vulnerable to food scarcity. Therefore, the mating season of honeybees generally depends on seasonal blooming cycles. Accordingly, in Sri Lanka (Koeniger and Wijayagunsekera 1976), Thailand (Rinderer et al. 1993) and Borneo (Koeniger et al. 1996b), swarming seasons of Asian honeybees overlap and sympatric *Apis* species produce drones and queens simultaneously. Because of the uniform mode of colony multiplication by swarming within the genus, there seems to be no “evolutionary flexibility” and the reproductive season among sympatric honeybee species is tightly synchronised. Another cause for similar reproductive seasons among honeybees seems to be the environmental requirements for mating flights. Especially in temperate conditions, mating flights are weather dependent. Strong winds, low temperatures and clouds are known to prevent mating flights of *A. mellifera* queens (Ruttner 1956a, b). Therefore, mating and swarming are restricted to a season when favourable weather conditions are most likely to occur.

6.15.2 Time of Mating Flights

6.15.2.1 The Allopatric Situation

Among the Asian honeybee species, *A. cerana* has the most extensive natural distribution. In consequence, it overlaps with many of the other Asian *Apis* species (Ruttner 1988). However, regionally, there are large areas where *A. cerana* is the only honeybee. Within the Asian continent, these areas are mainly in the northern part of their range, in mountain ranges and in the Japanese islands (with the exception of Hokkaido). A daily *A. cerana* mating period from 1230 to 1600 h has been reported from Bihar in North India (Sharma 1960). Drones of *A. cerana indica* (originating from the mountains of the North West Frontier Province of Pakistan) flew between 1200 and 1530 h in Germany (Ruttner 1973). Verma (1991) observed mating flights of *A. cerana indica* queens in the Shimla Hills (north India) between 1230 and 1530 h. In Japan, drones of *A. cerana japonica* flew from 1315 to 1700 h, and successful mating flights of *A. cerana japonica* queens occurred between 1435 and 1635 h (Yoshida et al. 1994; Yoshida 1995).

In all of the various places where *A. cerana* occurs as the only *Apis* species, drone flight periods last for more than 3 h and start shortly after noon. The time and overall duration of drone and queen flights and the timing during the early afternoon seem to be typical for honeybees in allopatric conditions. Under these conditions, the data are nearly identical in *A. mellifera* and in *A. cerana*.

6.15.2.2 The Sympatric Situation

The daily flight period differs under sympatric conditions with 2–4 indigenous *Apis* species. Then mating flights occur in a limited time slot, which is different across the species but may differ with different regions. *A. andreniformis* drones have a uniform flight period beginning after 1200 h and ending shortly before 1400 h in Borneo, peninsular Malaysia and Thailand as well (Rinderer et al. 1993; Koeniger et al. 2000). *A. florea* has a much wider distribution and the daily flight periods of drones show remarkable differences. In Sri Lanka, drones flew about 2 h earlier than in Thailand. According to Koeniger et al. (1989), the drone flight window of *A. florea* in Bangkok was between 1345 and 1530 h. In Chiang Mai, more than 97 % of the drones started after 1300 h and 93 % returned before 1530 h (Buawangpang et al. 2009). Southeast of Bangkok, in the overlap zone with *A. andreniformis*, the drone flight period occurred 30–45 min later and ended only at 1645 h (Rinderer et al. 1993). This resulted in an overlap with the *A. cerana* drone flight period for 90 min—the only case so far observed in a sympatric situation. The differences in the mating period of *A. florea* in Thailand may be explained with different times of the year or different weather conditions during data collection or with the presence of *A. andreniformis*.

A. cerana exhibits an even higher variability in drone flight periods. While in Sabah (east Borneo) it starts at 1400 h (Koeniger et al. 1994b), in Thailand (southeast of Bangkok) it starts shortly after 1500 h (Rinderer et al. 1993) and in Sri Lanka only after 1600 h (Koeniger and Wijayagunsekera 1976). In Sulawesi, the start of drone flight is the earliest with 1245 h (Hadisoelilo and Otis 1996). These differences cannot be explained by collecting the data at different times of a year. The drone flight period of *A. dorsata* seems uniform across Asia; drones start their flights shortly after sunset (Koeniger and Wijayagunsekera 1976; Koeniger et al. 1994c). Near the equator, the shift of sunset during the year is marginal. Further north (Nepal), the change of sunset time during the year exceeds an hour and the drone flight period is shifted accordingly (Woyke et al. 2001). Queens of all species only start for mating flights at the peak of the drone flight period thus maximising the chance to meet many drones and thus minimising the duration of their individual flights. The period of queen flights covers 30–45 min while it lasts generally more than 90 min for drones (except in *A. dorsata*).

6.15.2.3 Overlapping Habitats

Some *Apis* species have special habitats on mountains where an overlap with the lowland species occurs only in a thin borderline: examples are *A. nuluensis* and *A.*

cerana in Sabah, Malaysia and *A. laboriosa* and *A. dorsata* in Nepal. The open nesting giant honeybee species *A. laboriosa*, adapted to the high altitudes of the Himalayas, has a drone flight period between 1220 and 1420 h (Underwood 1990). Likewise, *A. nuluensis* is the only honeybee species in the mountains of Borneo above 1,700 m, and its drone flight period is between 1045 and 1315 h (Koeniger et al. 1996b). Drone flights were recorded only from two *A. nuluensis* and one *A. laboriosa* colony. There is need of additional confirmation.

6.15.2.4 Time Signals for Mating Flights

The isolated species-specific daily mating periods of sympatric Asian honeybees must be triggered by well-defined time signals. In *A. mellifera*, it is well documented that the internal clock of workers is synchronised on a colony level (Frisch and Koeniger 1994). Whether or not the signal for mating flight activity is based on internal signals of individual drones or queens or, alternatively, whether a general signal of the colony initiates the mating period was addressed by cross-fostering experiments. Sealed drone brood was partially exchanged between colonies of *A. cerana* and colonies of *A. koschevnikovi*, resulting in colonies with a mixed drone population: *A. cerana* and *A. koschevnikovi* drones were accepted in alien as well in conspecific colonies. Observations of drone flights showed a clear result. The drone flight periods of *A. cerana* drones and *A. koschevnikovi* from the same colony were significantly different. Drones of both species flew according to their species-specific periods whether or not they were kept in alien or conspecific colonies. Thus, the period of mating flight might not be determined by the colony. The internal clock of an individual drone seems to trigger the mating flight period (Koeniger et al. 1994b).

6.15.2.5 Duration of Individual Mating Flights

Across all species drones leave the colony for 20–30 min (*A. florea*, Buawangpang et al. 2009; *A. cerana*, Woyke 1975; Punchihewa 1992; *A. dorsata*, Tan et al. 1999; *A. koschevnikovi*, Koeniger personal observation). Individual flight duration seems independent of the size of queens or drones (Koeniger et al. 1993), of the flight speed (Koeniger et al. 2005) and even of the aerodynamic characteristics of the drones (Radloff et al. 2003). Therefore, we assume that the provisioning of the drones for the mating flight is regulated by the amount of food a drone can take before starting. The duration of individual queen flights is more variable and may last from 15 to 30 min (*A. koschevnikovi*, Koeniger et al. 1994a; *A. dorsata*, Tan et al. 1999; *A. cerana*, Woyke 1975; Punchihewa et al. 1990a, b). In *A. mellifera*, it depends mainly on the rate of successful copulations which queens could achieve (Schlüns et al. 2005; Koeniger and Koeniger 2007). We assume that the same mechanism holds true for the other species.

6.16 Drone Congregation Areas and Flight Behaviour of Drones

6.16.1 Drone Congregation Areas

It has been known for about 100 years that *A. mellifera* drones have a rendezvous place where they circle at a height of 10–30 m above distinct areas, the so-called drone congregation areas (DCAs) (review Koeniger and Koeniger 2000). DCAs were also detected for four Asian *Apis* species as well.

While *A. mellifera* drones assemble over open areas, drones of Asian species use prominent trees as landmarks for their DCA. The characteristics of the actual location at the trees vary among the species and, as known for *A. cerana*, even within its subspecies.

In Sri Lanka, *A. cerana indica* drones gather in close proximity to the trees. These drones restrict their flight to an open space near the canopies of trees and do not follow a (caged) queen far into the open space either above or at the side of the canopy (Punchihewa et al. 1990a, b). In Borneo, drones assemble (like in Sri Lanka) near canopies of trees (Koeniger et al. 1998). In Japan, however, drones of *A. cerana japonica* congregate in the open air high above prominent trees (Yoshida and Yamazaki 1993; Fujiwara et al. 1994). In Germany, *A. cerana* drones originating from northern Pakistan visited a DCA in an open valley (Ruttner et al. 1972; Ruttner 1973).

Taken together, DCAs of *A. cerana* show a high degree of variability. Thus, the specific features of these DCAs may be mainly the result of local adaptations to environmental factors. For example, avoiding predators, such as birds (*Merops* sp., etc.), by flying near tree canopies might have a higher selective advantage under tropical conditions than in the mountains of northern Pakistan. Considering the limited data available (in comparison to *A. mellifera*), we expect that an even wider range of differences among the DCAs of *A. cerana* may become apparent with ongoing research on Asian honeybees.

Drone congregations of *A. koschevnikovi* were regularly observed to occur under a thick cover of vegetation. The height above the ground of different DCAs varied between 1.5 and 12 m in the only region that has been studied, in the Tenom district (Sabah) (Koeniger and Koeniger 2000). At present, there is no information available on the DCAs of the other two cavity-dwelling honeybees, *A. nuluensis* and *A. nigrocincta*. In Borneo, drones of *A. dorsata* congregate under the canopy of tall emergent trees. The imminent, tall treetops seem to serve as visual landmarks, and applying this criterion, several “new” *A. dorsata* DCAs have been located (Koeniger et al. 1994c). The drones of *A. dorsata* assemble under the umbrella of the canopy and do not follow the dummy queen moved into the open air. Further, drone attraction showed a maximum of 3–5 m below the canopy. The height above the ground ranged between 10 and 35 m depending on the size of the tree. Recently, several *A. dorsata* DCAs have been detected under tall trees in Sri Lanka

6.16.2 Drone Competition at the Drone Congregation Area

Drones of most species (except *A. dorsata*) leave the colony twice or even three times per day and the mean of total flights are 13.6 days in *A. mellifera* (Rueppell et al. 2005) and 9.1 days in *A. florea* (Buawangpang et al. 2009). For a cohort of 20 *A. florea* males, all flights could be observed and the mean life flight time was summed up to 6 h and 38 min on 14.8 flights. There are no such data for the other species. But considering these data and the description of multiple flights per drone from other species (own observations) as a basis we hypothesise that the drones mean total life flight time of a species exceeds more than five times that of the queens' in the respective species. This hypothesis leads to the conclusion that the queen/drone ratio in a DCA is five times more biased towards drones than calculated for the ratio of brood cells. Calculating the reduction in virgin queens because of queen competition, the bias is even more enhanced. The extremely male-biased sex ratio at a DCA has resulted in a high competition among drones. The flight apparatus is well-developed (Radloff et al. 2003).

The sensory input is optimised towards fast queen recognition. The drone antenna has a large number of olfactory perception pore plates geared to detect queen pheromones. Drones of *A. mellifera* have about seven times more pore plates than worker bees (Esslen and Kaissling 1976). The huge compound eyes cover the whole upper part of the head. The number of ommatidia is much higher than in workers or queens and the spatial resolution is well-developed (Seidl 1980; Vallet and Cole 1993). These functional adaptations are more or less uniform throughout the genus *Apis* (Radloff et al. 2003). The adaptation of queens, however, to flight speed and detection of pheromones are, in comparison to their male counterparts, less expressed. Thus selective pressure caused by the male-biased sex ratio was focussed on the drones and queens remained more or less "unchanged".

When a single queen arrives at a DCA, it is crucial for a drone to recognise the queen quickly, faster than competitors. In many insect species, male insects are attracted by sex pheromones released by receptive females. The mode of orientation via pheromones is most effective when females are stationary and the male can follow the gradient of increasing odour. However, the situation at the DCA is different. During flight, the virgin queen changes her flight path constantly to stay within the DCA. Furthermore, drones fly near the queen wafting the pheromones irregularly. Thus, the sex pheromone will indicate the presence of a queen, but is not sufficient to detect the queen's position. For *A. mellifera*, it is known that visual cues play an important role in queen recognition (van Praagh et al. 1980; Vallet and Cole 1993; Gries and Koeniger 1996). *A. mellifera* drones follow butterflies or stones (Ruttner and Ruttner 1965) and similarly drones of *A. cerana* and *A. koschevnikovi* were observed to follow a hornet, *Vespa affinis* (Koeniger et al. 1994d). We cannot exclude that the hornet used additional cues such as odours to attract *A. cerana* drones because it was exploiting the drones' behaviour by catching a pursuing drone. The visual location of the queen may include various cues. From a more distant position, drones may recognise the dense group of drones following a queen and thus could be

attracted from a large distance. Video analysis of drone behaviour pursuing a tethered queen of three Asian species (Koeniger et al. 2005) revealed that the comet-shaped formation of drones in direct pursuit changed within seconds.

Further, often the group suddenly left the queen as if following a fellow drone. For orientation nearer to the queen colour plays a role. In *A. mellifera*, objects with different colours to those of a drone were more attractive. Further, queens marked with the bright white and orange mating sign (Koeniger 1990) attracted more drones than unmarked ones. Since drones of the Asian cavity-nesting species also produce mating signs (Koeniger et al. 2001), we assume a similar mechanism in these species.

Drones of the species *A. cerana*, *A. koschevnikovi*, *A. dorsata* and *A. mellifera*, which pursued a dummy queen moving in a circular course, flew in a comet-shaped formation below and behind the queen. The median numbers of drones in such a comet ranged from 9 (*A. koschevnikovi*) to 31 drones (*A. mellifera*). Video observations of competing drones of Asian species and *A. mellifera* did not reveal evidence for direct combat among drones chasing after a queen. Collisions of drones were rare and regularly resulted in the exclusion of both drones from the comet of pursuers. Thus, drone density behind the queen and distance to the queen has to be adjusted to avoid collisions between drones. The density of drones depends on their flight speed, which differs between the species and seems to be a limiting factor, which restricts the number of pursuing drones compared to the several hundreds or even thousands of drones present at the DCA. The median of flight speed near the queen ranged from 2.6 m/s (*A. koschevnikovi*) to 4.1 m/s (*A. dorsata*).

Competition of *Apis* drones seems to be focussed to fly fast in order to be the first when a queen is ready to mate and equally important to manoeuvre to the best position behind the queen and maintain it whenever she is still engaged in copulation. This seems to be difficult: the median duration of a drone's presence in the mating comet did not exceed 2 s. Either by overtaking or leaving/entering the comet, drones seem to compete for more promising positions in the pursuing comet. Good visual perception and the ability of high acceleration are necessary to reach and maintain such a position. Drones of all species can accelerate from 10 to 20 m/s². Drones keeping a position within a space of not more than 2,000 cm³ behind the queen were able to grasp the dummy and these successful drones often managed to stay longer near the queen than their fellow drones (Koeniger et al. 2005). *Apis* drones meet at a DCA in large numbers and fly their waiting loops before queens' start for the mating flights. The number of drones commuting daily between the colony and a DCA ranges from about 16,000 (*A. mellifera*) to several hundred in Asian species. The flight range per drone varies from 1 to 2 km for *A. cerana* and *A. koschevnikovi*, up to 5 km and more in *A. mellifera* (Ruttner and Ruttner 1966).

6.16.3 Internal Connection of Drone and Queen

Because of the initial docking, the flying drone places his genital opening into the open sting chamber of the flying queen. The soft tissue of the wall of the queen's sting chamber smoothly clings to the surface around the tip of the drone's abdomen.

Then with a sudden contraction of the abdominal muscles, the drone presses its haemolymph in the endophallus. The endophallus consists only of a thin chitinous membrane, which is densely folded, in the abdomen prior to copulation (Koeniger et al. 1991; Patinawin and Wongsiri 1993). The pressure from the haemolymph inflates the endophallus turning it inside out (eversion). Using only its own force, the drone can evert only half of the endophallus, then he becomes paralysed and the motionless body loses its grip and is forced about 90° backward by the form of the inflated endophallus (Koeniger 1986). In this phase, the half-everted endophallus terminally forms a thin tube (cervix) which contains the sperm, while the basal part is extended by large masses of mucus and firmly fills the queen's sting chamber and vagina like a cork in the neck of a bottle. The anchorage of the paralysed drone is probably further supported by the queen's complex system of muscles consisting of several ring (sphincter) and longitudinal groups of muscles at the vagina and median oviduct and a conspicuous muscular valve-fold in the vagina (Camargo and Mello 1970). The short, thick cornua of the endophallus with its "orange coloured" sticky and greasy secretion may further contribute to strengthen the attachment. In the honeybees, transfer of spermatozoa and mating sign is more complicated because of the double function of the endophallus—as a coupling element and as well as a transfer system of sperm and mating sign.

6.16.4 *Sperm Deposition*

The drone is immobile after insertion of the half-everted endophallus and is no longer in command of its muscles. Thus, the sperm transfer and separation of the pair is initiated by the queen and it happens within a split second. The queen contracts her genital muscles and those of the sting chamber. First, the sperm transfer from the thin tubular cervix into the oviducts is completed and the oviduct is probably closed by the vaginal valve-fold.

Almost at the same time, the full eversion of endophallus and cornua follows with dramatic effects. In the cavity-nesting species, the mucus is still inside the endophallus and firmly fills the sting chamber. The thin membrane of the endophallus is peeled off during full eversion. First, the thin tubular cervix opens its folds by unzipping the initial hairy breech (Woyke 2008) and thus can glide over the mucus, followed by the last part of the endophallus, the bulb. At the same time, the cornua are fully everted and push the endophallus (with the immobile drone!) out of the queen, leaving their sticky orange secretion together with the mucus in the sting chamber, the so-called mating sign. In conclusion, the above procedure does not involve a rupture of the drone's endophallus. In *Micrapis*, decoupling of the pair seems easier. The queen turns the hind leg in a way that the thumb-like clasp is detached. Simultaneously, she loosens the grip of the endophallus with her genital muscles and the drone will fall down. Only the cornual secretion is left in the sting chamber (Koeniger et al. 2000). A different mechanism must be used by *A. dorsata*. Uncoupling the hind legs of the drone from the queen's abdomen may be achieved

by pushing them off with the queen's hind legs. In the process of full eversion, the long curled cornua may push the drone back. It cannot yet be decided if the mucus is left in the sting chamber similar as in *A. mellifera* or if it will be removed together with the endophallus. In five queens returning from 14 mating flights, no mating signs were seen (Tan et al. 1999). During the experimentally induced eversion of the endophallus, no mucus was expelled (Woyke 2001).

In *Apis*, endophalli do not rupture during the uncoupling of the pair. Thus, the detachment process must not be understood as an autotomy (like the sting). Sperm, and later male gland secretions, are transferred to the queen. However, the drone will die after falling to the ground because its haemolymph remains irreversibly in the everted endophallus. Returning to the initial comparison, undocking of a space shuttle is principally different from the separation of the drone from the queen. Before a shuttle separates from the station the internal connection is hauled inboard, the locks are closed and only then are the external connections detached. Many space shuttles can be reused several times; the drone, however, is a kind of "one way shuttle", once his sperm is transferred, he is bound to die. As discussed earlier, this might be due the male-biased sex ratio in honeybees.

6.16.5 Mating Sign

During copulation, drones transfer in addition to sperm some structured secretions, the mating sign, which is deposited in the queen's sting chamber. Queens of cavity-nesting species return from mating flights with a mating sign that protrudes from the sting chamber. It is generally removed only after entering the nest by rubbing the abdomen on the comb or with help by workers and is carried out of the hive within a couple of minutes after return. These mating signs were described for *A. mellifera* (Woyke and Ruttner 1958), *A. cerana* (Woyke 1975) and *A. koschevnikovi* (Koeniger et al. 1994a). In the free-nesting dwarf honeybee *A. andreniformis*, the structure of a mating sign does not contain white mucus but only two stripes of an orange-coloured secretion (Koeniger et al. 2001). In *A. florea* (Koeniger et al. 1989) and *A. dorsata*, no male secretions were detected in the queen (Tan et al. 1996) though *A. dorsata* drones have well developed mucus glands (MG) in both species, the endophalli produce the orange-coloured corneal secretion. To explore the composition of mating signs of *Apis* species, it is helpful to look at the anatomy and structure of endophalli (Figs. 6.9, 6.10 and 6.11). The mucus is produced by the male accessory glands (mucus glands MG), the connective substances connecting the chitin plates in *A. mellifera* originate from the bulbus gland and the orange-coloured secretions from the cornual glands (CG) (Koeniger et al. 1989; Koeniger et al. 1996a). MG is well developed in all drones of the hive-nesting species and in *A. dorsata* (Koeniger et al. 1991): For the male reproductive organs of *A. laboriosa*, no differences from *A. dorsata* were found (McEvoy and Underwood 1988). By contrast, in *A. florea* and *A. andreniformis*, MG is tiny. Likewise, in these species, the bulbus is not as thick as in the others. According to these data, a mating sign composed of mucus and secretions of the

bulbus and cornual gland are formed by *Apis* and maybe also by *Megapis* species. However, the bulbus gland is not yet studied in detail and the function of its secretion is not yet understood. We suggest that it may form a tube like structure enclosing the soft mucus.

Until 1950, the mating sign was interpreted as a plug to prevent further mating. The function as a mating plug can be set aside. First, it does not prevent multiple mating during one flight. For *A. mellifera*, several matings during one flight were proven by Woyke (1960), for *A. cerana* by Woyke (1975) and for *A. koschevnikovi* by Koeniger et al. (1994a). At the end of mating, every drone leaves its own sign (Woyke and Ruttner 1958; Koeniger 1986). In *A. mellifera*, drones remove the mating sign by pulling it out with a special hairy field of the endophallus. This hairy field is also present in the species of the subgenus *Apis*. Queens of *A. cerana* and *A. koschevnikovi* also return from one mating flight with a mating sign and their oviducts are filled with the sperm of about 5–10 drones (Koeniger et al. 1994a). Obviously, drones of these species too are able to remove the mating sign of their predecessors.

Second, the mating sign does not prevent sperm loss. Queens of *A. mellifera*, *A. cerana* and *A. florea* have a muscular vaginal valve (valvula vaginalis, Camargo and Mello 1970; Camargo 1972; Ruttner et al. 1973) which actively closes (or opens) the oviduct (Ruttner 1956a, b) and thus regulates the rejection of sperm. Further, mating signs in *A. mellifera* are removed within seconds during mating flights by drones and only few minutes after the last mating in the colony while the filling of the spermatheca takes several hours (Woyke 1988). A similar mechanism may function in the other species of the subgenus *Apis*. In *Micrapis*, the sperm is deposited at the orifice of the spermatheca to the spermatheca by the pointed endophallus. Here the valvula vaginales in addition may regulate the positioning of the male copulation organ. In *Megapis*, such a valvula vaginalis is not present. As mentioned above, the function of mating signs was long debated. Much later, video recordings demonstrated that drones (*A. mellifera*) remove the mating sign by pulling it out with a special hairy field of the endophallus (Koeniger 1986).

However, the debate about the purpose (adaptive advantage) of the mating sign—this “costly” drone product—is still in progress and some arguments shall be presented here. A major problem of drones in the middle of a large group of fast moving competitors is to recognise a fast-flying young queen. Attempts of drones to copulate with fellow drones occur regularly. Based on these observations, the idea was put forward that the mating sign might mark the young queen and facilitates queen recognition for successive drones. A set of experiments supported the queen recognition hypothesis. Dummy queens within attached mating sign were significantly more attractive than the control dummies (Koeniger 1990). However, why should a successful dead drone support his successor? First, after mating the queen carries the sperms of the previous drone. In this situation, selection will eliminate drones whenever the queen with “his” sperm does not reach the safety of the colony. Drones, however, would benefit whenever they succeed to reduce the duration of queen’s risky mating flight. An obvious way to “end” the queen’s flight would be to seal her vagina. Apparently, the mating sign does not prevent further mating (as mentioned earlier). Why? The answer is the high adaptive value of the genetic

diversity within the *Apis* colony and evolution has favoured extreme polyandry of *Apis* queens. Drones benefit by supporting the next male to mate the queen in fast succession. Thus, after being successful in the competition with fellow drones, a drone seems to cooperate “post-mortem” with his successor by marking the queen with a conspicuous mating sign in order to reduce the mating-flight time. In *A. cerana* and *A. koschevnikovi* and even in *A. andreniformis*, parts of the mating signs protrude out of the sting chamber (Koeniger et al. 1994a, 2001).

6.16.6 *Sperm Transfer*

In the genus *Apis*, total numbers of spermatozoa transferred to the queen from an average of 1.7 million in *A. andreniformis* to an average of 107 million spermatozoa in *A. mellifera*. The numbers of spermatozoa in the queen’s spermatheca vary from an average of 0.78 million in *A. andreniformis* to 4.73 million in *A. mellifera*. So the ratio (of total spermatozoa deposited in the queen) reaching the spermatheca is about 50 % in *A. andreniformis* and less than 5 % in *A. mellifera*. Thus, within the genus *Apis*, spermatozoa deposition by drones and the transfer of spermatozoa within the genital system of the queen vary significantly among the species, which is the topic of this section.

6.16.7 *Sperm Deposition by the Drone*

The endophalli of *Apis* drones can be classified into three types. In the subgenus *Apis* (cavity-dwelling species), sperm is ejaculated when the short cervix is still a thin tubule and spermatozoa are brought into the median and lateral oviducts behind the vaginal valve (Fig. 6.9). The queen probably actively bends the valve-fold and also must relax the sphincter-like muscles of the median oviduct. Mucus only appears later during eversion of the bulbus. In the *Megapis* drones, the cervix of the endophallus is extremely long and spermatozoa are ejected through the cervix before the eversion of the bulbus starts (Woyke 2001). The long cervix of the *Megapis* endophallus probably reaches the lateral oviducts during ejaculation of spermatozoa. Also, in *Megapis*, the excretion of mucus is linked with the eversion of the bulbus, which occurs at the last stage of the eversion of the endophallus. Thus, in both subgenera (*Apis* and *Megapis*) during natural mating excretion of mucus is temporally separated and occurs after sperm deposition. In *Micrapis*, the mode of sperm deposition is strikingly different. The spermatozoa are ejaculated only after the complete eversion of the endophallus at the pointed tip of the tubular bulbus. Further, a more active reaction of the queen’s genital muscle system may play an important role to allow placing the tip of the endophallus exactly at the orifice of the spermathecal duct.

6.16.8 *Distribution of Individual Sperm Portions*

In the subgenus *Apis*, sperm of the following drones will push the sperm of their predecessors further into the lateral oviducts. However, the sperm of different drones in the oviducts does not remain in different well-defined layers as described by Moritz (1993). The walls of oviducts are not solid (like a glass syringe for instrumental insemination). Instead, the walls of oviducts are thin, flexible and folded. Thus, the oviducts are actually extended by the sperm. In addition, the two lateral oviducts may be filled by different drones. A simple model was used to simulate the physical conditions for sperm transfer. A small rubber balloon was consecutively filled with differently coloured portions of a semi-fluid (with a consistency similar to honeybee sperm). As a result, portions were neither layered nor evenly mixed, but each coloured portion was distributed unevenly, sometimes as a compact part in the centre, sometimes as a stretched mass along the walls. The first red portion was displaced to the margin, while the last and green portion is mainly in the centre. According to the above model, the sperm bundles of the drones are also unevenly distributed in the subgenus *Apis* and in *Megapis* as well.

In conclusion, because of the dilatibility of the lateral oviducts, the position of the sperm bundles per drone is incidental and neither a regular first nor a last male have a preferential place for the filling process of the spermatheca. This is in line with the results of Franck et al. (2002) who could not find a last male advantage in naturally mated queens of *A. mellifera*.

6.16.9 *Transfer to the Spermatheca*

The anatomical structure of the queen's genital duct plays an essential role during sperm transfer. *A. mellifera*, *A. cerana* and *A. florea* queens possess a bursa copulatrix and a complicated muscle system stretching from the vagina to lateral oviducts. It consists of (1) a muscular valvula vaginales, (2) several muscles inserting at the vagina and (3) a muscle surrounding the median oviduct cranial of the orifice of the spermathecal duct. The genital duct of giant honeybee queens differs from the other species. Queens of *A. dorsata* lack a bursa copulatrix as well as the muscular system of the vagina and median oviduct, and the valve-fold has no muscles (Camargo and Mello 1970; Camargo 1972). We suggest that the queens, which have been studied, represent the typical character for each of the three subgenera of honeybees.

The transfer of spermatozoa from the oviducts to the spermatheca has only been studied in detail in *A. mellifera* (Ruttner and Koeniger 1971; Gessner and Ruttner 1977). Obviously, this process is a combination of active movements of the queen's muscles and motility of the spermatozoa. Spermatozoa of *A. mellifera* as well as those of *A. cerana* and *A. dorsata* have the tendency to accumulate with their heads against walls (personal observation). During the transport of the sperm from the oviducts in the direction of the vagina by the muscles of the lateral oviducts, some spermatozoa reach the orifice of the spermathecal duct. Then the muscular system

of the spermathecal duct (Bresslau's sperm pump) and the valvula vaginalis play a crucial role. The former sucks the spermatozoa into the spermatheca and the latter regulates the ejection of superfluous spermatozoa.

In cavity-nesting honeybee species, all spermatozoa which accumulate at the dorsal side of the oviducts will have a chance to enter the spermathecal duct during their transportation back to the vagina. Spermatozoa positioned in the centre of oviduct or even further away from the orifice of the spermathecal duct are ejected through the vagina. This mechanism may explain how small bundles of spermatozoa of many mates are present in the spermatheca. The differences per drone may result from the specific position of the respective sperm bundle. In *A. cerana* and *A. koschevnikovi*, about 9 % of the spermatozoa reach the spermatheca. In *A. dorsata*, the percentage is only 5.5 % though drones have about the same number of spermatozoa. This low percentage may be explained by the missing muscular system of the genital ducts and by the large spermatheca which can hold up to 4 million spermatozoa compared to 1.4–2.1 in *A. cerana* and *A. koschevnikovi*.

The filling process is different in the dwarf honeybees. The number of spermatozoa per drone is low and obviously the deposition at the orifice of the spermathecal duct allows an instant migration into spermatheca before the next mating (Koeniger et al. 2000). Accordingly, a much higher percentage of spermatozoa per drone (*A. florea* 31 % and *A. andreniformis* 45 %) reach the spermatheca. To which extent the sperm is displaced by sperm of the next drone at the orifice before it can enter the spermatheca is not known. Maybe because of the higher number of spermatozoa in *A. florea* only a lower percentage of spermatozoa can enter the spermaduct than in *A. andreniformis* before it is displaced by the endophallus and sperm from the next drone. In any case, queens of the dwarf honeybee have many spermatozoa in their spermatheca when they return from mating flights (Koeniger et al. 1989, 2000).

6.16.10 Sperm Storage

In the genus *Apis*, queens store living spermatozoa for several years. Queens provide specialised morphological structures for sperm storage. The spermathecae are equipped with a huge gland and surrounded by a dense tracheal net. Amazingly, little is known about how queens are able to keep sperm cells viable over such a long period of time. Research has started only for *A. mellifera*. Glandular secretions, produced by the spermathecal glands, are found in the spermatheca (Klenk et al. 2004). These secretions contain proteins, metabolites and other chemicals. In *A. mellifera*, several proteins have been proposed to be responsible for this storage (Collins et al. 2006). In addition, high K⁺ concentrations and the high pH of the spermathecal fluid have been proposed to lower the metabolic rate of sperm during storage (Verma 1973). A systematic analysis of these female-derived proteins has been conducted recently combined by a proteomic identification of these proteins and their metabolic network (Baer et al. 2009). The technique of instrumental insemination has permitted the study of heterospecific sperm transfer and storage. The longevity was reduced within days when the cross-inseminated pair was composed of different subgenera.

6.17 Polyandry of Honeybee Queens

Polyandry of queens in the genus *Apis* was discovered applying several different classical methods:

1. Offspring analysis of genetically marked queens and drones (Roberts 1944; Taber 1954 and others).
2. Comparison of sperm volumes of drones with the sperm volumes in the oviducts of queens returning from mating flights (Triasko 1956a, b; Woyke 1960).
3. Determination and frequency of diploid drones in a population (Adams et al. 1977). The estimations with these methods ranged from 7 to 17 successful matings per queen. The use of modern, molecular methods started only about 20 years ago and resulted in a similar range of mating frequencies. For the SE Asian species two methods have mainly been used—comparisons of sperm numbers in freshly mated queens with sperm numbers of drones or using DNA micro-satellites. Sperm counting allowed an estimation of the minimal number of mating of queens per mating flight. The total number of patrines per queen can be determined only by analysing the paternity frequency of a colony using DNA micro-satellites. A review on eight species of *Apis* was published by Tarpy et al. (2004), Paar et al. (2004), Oldroyd and Wongsiri (2006) and Takahashi et al. (2008). Differences in mating frequencies were widely accepted as typical for each species in spite of small colony sample sizes and restricted sampling sites. Thus far (2010) only four colonies of *A. cerana* (Oldroyd et al. 1998), *A. koschevnikovi* (Rinderer et al. 1998) and *A. nigrocincta* (Otis and Hadisoeso 1996) from a single site were analysed. Recently, more data were published for *A. andreniformis* and *A. dorsata*. Only from the European species *A. mellifera*, more than 100 colonies were studied, but mainly from honeybees living under temperate conditions. The published sample size of *A. mellifera* is about 123 with an overall mean effective paternity frequency of 12. Splitting the original data of different samples reveals a large variance in queen-mating within the same locality among colonies. It is discussed that honeybee queens modulate their number of nuptial flights according to the environmental conditions which is supported by data of Neumann et al. (1999): in *A. mellifera carnica*, the effective paternity frequency averaged to 20.4 ± 1.7 ($n = 16$) on the mainland but was only 11.8 ± 1.2 ($n = 13$) on an island. The large genetic variance for polyandry in honeybees and its high heritability further indicates that polyandry may be favoured by balanced selection between individual queen and colony level (Kraus et al. 2005a, b). Also significant differences in their effective paternity frequencies were demonstrated on a subspecies level (Franck et al. 2000). They range from 9.3 ± 3 in *A. mellifera sicula* to 34 ± 14.2 in *A. mellifera capensis* and according to Kraus et al. (2004) 21.1 ± 8.2 in *A. mellifera carnica* and 40.9 ± 14.1 *A. mellifera capensis*. Significant differences also occurred in studies of *A. dorsata* in different regions. While in north and central Thailand, the effective mating frequency was 63.0 ± 5.7 ($n = 13$) (Watanachaiyingcharoen et al. 2003), it was 25.6 ± 11.6 ($n = 6$) in Borneo (Malaysia)

(Moritz et al. 1995) and 20.0 ± 6.6 in south eastern part of Thailand ($n = 4$) (Oldroyd et al. 1996). A similar situation was found in recent studies with *A. andreniformis*: the effective mating frequency of queens in the population from the Malay Peninsula (Takahashi et al. 2008) and the Thai population of this species (Oldroyd et al. 1997) were 11.7 and 10.5, respectively, differing significantly from queens in the Borneo population with 17.6 effective mating frequencies (Takahashi et al. 2008).

Including recent results (until 2009) in most *Apis* species, the number effective paternity ranges from about 12–18, with one exception in each of the main groups. *A. florea* have lower (7.9 ± 3.33) and *A. dorsata* have higher (44.2 ± 27.15) frequencies and the cavity-dwelling species *A. nigrocincta* (40.3 ± 23.4). These data suggest that the high level of patriline is a common trait for the genus *Apis* and is modified only by one species per sub-genus. Thus, we suggest that hypotheses for the evolution and maintenance of extreme polyandry in *A. mellifera* also hold good for the Asian species. Several hypotheses are published, such as sperm need, task specialisation, sperm selection, reproductive conflict, resistance to parasites and diseases (Sherman et al. 1988; Palmer and Oldroyd 2000; Tarpy and Page 2001; Oldroyd and Wongsiri 2006). Schlüns et al. (2005) re-analysed published data by applying a sample size calibration method to survey the differences and similarities in paternity skew among seven species in the genus *Apis*. The frequent patriline appeared to be similarly distributed in all tested species. The rare patriline is those which contribute most to the deviation from homogeneity. Omitting the rare patriline in the correspondence analysis, the differences of frequency distribution of the nine most frequent patriline were no longer significant. Thus, there is an astonishing similarity in the most frequent patriline, in spite of the significantly different mating frequencies and the different modes of semen transfer in the dwarf honeybees and different numbers of spermatozoa in drones from 0.14 to more than 10 million (Koeniger and Koeniger 2000; Palmer and Oldroyd 2000).

The paternity skew among honeybee species still differs significantly, particularly due to the rare patriline. Fuchs and Moritz (1998) suggest that some genes for any worker specialisation confer an advantage on colony fitness only when they are rare. This would require a stable mix of sperm from a few drones which contribute that trait and many which do not. To ensure both specific, low within-colony proportions of “rare specialist” genes and to reduce random variation of these proportions would require mating with high numbers of drones. The quantitative implementation shows that moderate to very high numbers of matings are required to exploit colony advantages from genotypic allocation of workers to rare tasks. Extreme polyandry could thus result from colony selection dependent on the intra-colonial frequency of rare genetic specialists. In any case, the high polyandry of queens is a remarkable common character of the genus *Apis* which results in a very low genetic relatedness of 0.27–0.29 among workers within one colony.

6.18 Reproductive Isolation

Achieving reproductive isolation becomes an evolutionary ‘key event’ which marks a point of independent genetic development and divergence. In honeybees, research into reproductive isolation has had a relatively recent start. The Western honeybee species *Apis mellifera* L. with its wide, natural distribution in Europe, in the Middle East and Africa, and its successful introduction into the Americas and Australia has dominated scientific interest. This ‘absence’ of reproductive isolation lasted until 1965, when *A. cerana Fabricius* colonies from China were imported into Germany, and the first pioneering research on reproductive isolation among honeybees was initiated by F. Ruttner. In contrast to *A. mellifera* drones which congregate in the open air, *A. cerana indica* drones in Sri Lanka and in Borneo gather in close proximity to the trees. These drones restrict their flight to an open space within or near the canopies of the trees. They do not follow a (caged) queen far into the open space above or at the side of the canopy (Koeniger et al. 1998, Punchihewa et al. 1990a, b). The distance between the drone congregation area and the drone colonies is clearly less than in *A. mellifera* and ranges up to 2 km (Tingek, unpubl. data). In Japan, however, drones of *A. cerana japonica* congregate in the open air high above prominent trees (Fujiwara et al. 1994; Yoshida and Yamazaki 1993). In Germany, *A. cerana indica* drones originating from Northern Pakistan visited a drone congregation area in an open valley far away from the trees (Ruttner 1973; Ruttner et al. 1972).

Since these differences can occur within the same subspecies (*A. cerana indica*), the specific features of these drone congregation areas may be mainly the result of local adaptations to environmental factors. For example, avoiding predators such as birds (*Merops* sp., etc.) by flying near or within a tree canopy might have a higher selective advantage under tropical conditions than in the mountains of Northern Pakistan (Punchihewa 1992). Considering the limited data available (in comparison to *A. mellifera*), we expect that an even wider range of differences among the drone congregation areas of *A. cerana* may become apparent with further research on Asian honeybees. Drone congregations of *A. koschevnikovi* were regularly observed to occur under a thick cover of vegetation, and the height above the ground of different drone congregation areas varied between 1.5 and 12 m (Koeniger et al. 1998). At present, there is no information available on the drone congregation areas of the other two cavity-dwelling honeybees, *A. nuluensis* and *A. nigrocincta*.

In Borneo, drones of *A. dorsata* congregate under the canopy of tall emergent trees. It is not surprising that among allopatric species, drone congregation areas show similarities. Some convincing evidence for these similarities came from Ruttner (1973). In Germany, drones from imported *A. cerana indica* colonies (which originated from Pakistan) were caught together with simultaneously flying *A. mellifera carnica* at the same drone congregation areas.

A few *A. koschevnikovi* drones were caught at the optimum of the *A. cerana* drone congregation area and at the *A. dorsata* drone congregation area. *A. cerana* drones were caught at the *A. dorsata* drone congregation area, but not at the *A. koschevnikovi* drone congregation area. *Apis dorsata* drones came to the drone congregation area of

A. cerana, but not to the densely covered drone congregation area of *A. koschevnikovi*. As a result of these experiments, single drones were attracted and copulated at a drone congregation area of another species (with the exception of the *A. koschevnikovi* drone congregation area).

The major active chemical component of the *A. mellifera* queen's mandibular gland was identified as (*E*)-9-oxo-2-decenoic acid (9-ODA) by Callow and Johnston (1960). Among several other important biological functions, 9-ODA was found to be the main component of the *A. mellifera* queen's sex attractant (15, 59). Later, it was demonstrated that extracts of queens of three other *Apis* species (*A. florea*, *A. cerana* and *A. dorsata*) attract *A. mellifera* drones, and that these extracts contained 9-ODA. *A. dorsata* and *A. cerana* queens had a quantity (150–300 µg) of 9-ODA similar to that of the *A. mellifera* queen (Butler et al. 1967; Sheppard 1999). Consequently, dead, extracted queens or black dummies of similar size are the mating signs of an *A. mellifera* drone (easily recognised by its chitinous plate). This is—as far as we know—the only direct evidence of heterospecific mating of a free-flying *Apis* queen!

6.18.1 Different Daily Mating Periods

As described earlier mating among different sympatric honeybee species does not occur. The separation of species-specific daily mating periods blocks the meeting of alien sex partners. But what were the environmental requirements for a selective shift of the daily mating period? In general, the daily time of mating flights is subject to natural selection, too. Leaving the safety of the nest for mating poses a risk for the queen and for the colony as well. Selection should favour queens which fly at the most convenient (warmest?) and stable weather conditions. At least in the northern regions of Asia and on higher mountains obviously noon is the time with the best mating flight conditions. In these areas, *Apis* mating always occurs in the early afternoon. The same is true for all races of *A. mellifera* in temperate zones. In conclusion, under temperate climatic conditions a shift of the mating period towards daytime with suboptimal weather conditions may have a highly negative effect because of high queen losses. Inconsequential under those climatic conditions, the survival of honeybees may depend on an optimal daily mating period and time-sharing mating periods may not be possible. Thus the occurrence of sympatric *Apis* species seems to require warm weather conditions and a relatively constant day length. This is true for southern Asia with its tropical and subtropical climatic conditions, and accordingly sympatric *Apis* species are restricted to those regions. Tropical Africa, however, has been reached only by *Apis mellifera* more recently (Ruttner 1988).

6.19 Heterospecific Transfer and Storage of Spermatozoa

The occurrence of further reproductive isolation mechanism reflects the independent development of two species. The technique of instrumental insemination permitted the study of heterospecific sperm transfer and storage. *A. mellifera* queens were

inseminated each with about 8 million spermatozoa from *A. mellifera*, *A. cerana*, *A. dorsata* or *A. florea* drones. The number of spermatozoa reaching the spermatheca was the same. These data suggest that either physiology of the genital duct and its fluid is similar across all tested species or the drones' seminal fluid contains enough substances which enable the spermatozoa to reach the spermatheca. Within the next 4 weeks, motility of spermatozoa of *A. mellifera* and *A. cerana* did not change and remained at nearly 100 %. The motility of *A. florea* spermatozoa decreased to 33.9 % and motility of *A. dorsata* spermatozoa decreased to 26 % after 4 weeks (Phiancharoen et al. 2004).

In the case of closely related (sister) species, after cross-insemination of *A. cerana* queens with sperm of *A. koschevnikovi* and vice versa (Koeniger and Koeniger 2000; Phiancharoen unpublished data), spermatozoa entered the spermatheca at about the same percentage as after natural mating (Koeniger and Koeniger 2000; Palmer and Oldroyd 2000). In all cross-inseminations between *A. cerana* and *A. koschevnikovi*, spermatozoa in the spermatheca were viable when queens were dissected 40 days after insemination. The physiological conditions in spermathecae of cavity-nesting species enabled only a short survival spermatozoa of the dwarf and giant honeybees.

6.20 Fertilisation of Eggs and Hybrids (Post-Zygotic)

In all tests of cross-insemination across *Apis* species, the embryo died or no reproductive hybrids were obtained. Ruttner and Maul (1983) cross-inseminated queens of both *A. cerana* and *A. mellifera* successfully, but no larvae developed from the eggs. Histological studies revealed that the eggs were fertilised. During the first 3 days of development, the cells disintegrated and the embryo died during the blastoderm stage. No offspring emerged after insemination of *A. mellifera* queens with sperm of a representative species of each of the three subgenera. Calculations based on non-hatching eggs showed that at the beginning of oviposition the same percentage of *A. mellifera* eggs were fertilised by spermatozoa from *A. cerana* and *A. florea* while it was much less by *A. dorsata* spermatozoa (Phiancharoen et al. 2004). This was different after the inter-specific insemination of seven *A. cerana* queens with *A. koschevnikovi* sperm. In all cases, larvae developed. We could study the emerged offspring from only two queens. In both cases, we obtained hybrids with mixed drone- and worker-like characters (Koeniger and Koeniger 2000).

In conclusion, sperm longevity in alien spermathecae, successful fertilisation of eggs and finally the grade of development of hybrids reflect the genetic distance and follow the phylogenetic tree. Complete reproductive isolation under sympatric conditions is achieved in a very early stage and resulted in avoiding heterospecific contacts, and this is based on separated mating periods. Importations of allopatric honeybee species regularly result in heterospecific copulations with deadly consequences.

6.21 Reproductive Isolation Due to Allopatric Situation

Among the Asian honeybee species, *A. cerana* has the most extensive natural distribution. In consequence, it overlaps many of the other Asian *Apis* species (Ruttner 1988). Regionally, there are, however, large areas where *A. cerana* is the only honeybee. Within the Asian continent these areas are mainly in the northern part of their range, in mountain ranges and in the Japanese islands (with the exception of Hokkaido). A daily *A. cerana* mating period from 1230 to 1600 h has been reported from Bihar in North India (Sharma 1960). Drones of *A. cerana indica* (originating from the mountains of the North West Frontier Province of Pakistan) flew between 1200 and 1530 h in Germany (Ruttner et al. 1973). Verma (citealpCR199t) observed mating flights of *A. cerana indica* queens in the Shimla Hills (North India) between 1230 and 1530 h. In Japan, drones of *A. cerana japonica* flew from 1315 to 1700 h, and successfully mated (Yoshida et al. 1994). The mating period of *A. mellifera* and the observations from regions where *A. cerana* occurs as the only *Apis* species shows a striking degree of similarity. The overall duration of drones' and queens' flights and the timing during the early afternoon seems to be nearly identical in *A. mellifera* and in 'allopatric' populations of *A. cerana*. The drone flight period of *A. cerana indica* drones in Sri Lanka was confirmed by Punchihewa et al. (1990a, b). Accordingly, queens successfully mated between 1615 and 1655 h (Punchihewa 1992; Punchihewa et al. 1990a, b). The mating period of *A. cerana indica* in Sri Lanka is the latest so far recorded for this species.

A. koschevnikovi drones fly during a long period of nearly 2 h. Queens flew between 1700 and 1815 h (33). *A. dorsata* fly consistently at sunset. The flight period of *A. dorsata* drones is very short. Drones of this species perform only a single daily flight (Koeniger et al. 1994a, 1994b, 1994c, 1994d). In Borneo, a slight overlap with *A. koschevnikovi* drones occurred. This was, however, too slight to affect the reproductive isolation.

Koeniger and Wijayagunsekera (1976) observed that *Apis florea* in Sri Lanka occupies the time window which is nearest to the 'allopatric' mating period. Therefore, they argued that *A. florea* was the original *Apis* species to arrive at Sri Lanka. Consequently, the species with later periods would have established colonies in Sri Lanka some time later. With the evidence available today, however, a general and uniform pattern related to taxonomy becomes apparent: the first position directly after noon is held by a dwarf bee species (*A. Andreniformis* and/or *A. florea*). The next time window seems to belong to one or even two cavity-dwelling species (*A. cerana* and *A. koschevnikovi*); and at the very end of the day, just around sunset, *A. dorsata* holds. *A. cerana* occurs as the only *Apis* species which shows a striking degree of similarity. The overall duration of drones' and queens' flights and the timing during the early afternoon seems to be nearly identical in *A. mellifera* and in 'allopatric' populations of *A. cerana*.

6.22 Reproductive Isolation Due to Different Size

A well-known example for size differences as a copulatory barrier comes from domestic animals. For example, females and males of different dog breeds are attracted to each other, but because of differences in size are unable to copulate. In *Apis* species, worker bees vary considerably in size (Seeley 1985). The weight relation of workers of the dwarf honeybee species and the Asian cavity-dwelling species is about 1–2.5; to the giant honeybees it is about 1–5 (Koeniger et al. 1993). But the difference in weight in drones in these species is much smaller. The relation between the dwarf and the Asian hive-dwelling bees increases gradually to 1.5, and compared to the giant honeybees it is about 1–2. Only the drone of the allopatric *A. mellifera* has a considerably higher weight. The weights of queens have about the same relation as in drones.

As previously mentioned drones of all species will grasp the same queen dummy and try to copulate. Considering the small size differences (especially within the same taxonomic group), we conclude that body size ‘per se’ does not play a major role as an isolation mechanism in honeybees. However, it might be an important factor for the positioning of a specific mating period within the afternoon techniques. They introduced drones of *A. koschevnikovi* and *A. cerana* into alien colonies. As a result, *A. Koschevnikovi* drones flew during their species-specific mating period independently of their *A. cerana* host colony. Similarly, *A. cerana* drones followed their own mating period and not the *A. koschevnikovi* colony’s timetable. Later, virgin *A. koschevnikovi* queens were introduced into *A. cerana* colonies and flew at their own species’ mating period (Koeniger et al. 1996a, 1996b, 1996c). Drones and queens seem to decide on the right time for mating on the basis of an ‘inherited timetable’. Consequently, a direct evolutionary impact on the individual drone or queen and selection for changes in mating period becomes operational and may act faster than any effect via the colony (workers). Thus, fast adaptations to predatory pressure, to other environmental alterations or even to ‘new’ honeybee species are facilitated.

6.23 Physiological Barriers

6.23.1 Sperm Transfer

The percentage of drone spermatozoa stored in the spermatheca is quite different in different species, although DNA studies have revealed that the number of effective matings is similar among species (Estoup et al. 1994; Moritz et al. 1995; Oldroyd et al. 1995; Oldroyd et al. 1997, 1998; Rinderer et al. 1998). The total number of spermatozoa in the queen’s spermatheca divided by the effective number of matings indicates the amount of spermatozoa contributed by each drone. For example, an *A. mellifera* drone produces about 12.7 million spermatozoa, but only about 370,000

reach the spermatheca. Although this is the highest number for all species, it corresponds to only about 3 % of the spermatozoa of a single drone. This number is around 10 % in *A. koschevnikovi*, *A. cerana* and *A. dorsata*. The other extreme occurs in *A. andreniformis*: one drone produces 0.13 million spermatheca, of which an average of 66 % is present in the spermatheca (Koeniger et al. 1990). These 1.9 million living spermatozoa were counted in the spermatheca of *A. cerana* (Ruttner and Maul 1983). In the inter-specific and intra-specific inseminations of *A. koschevnikovi* and *A. cerana*, about the same percentage of spermatozoa (8–9 %) reached the spermatheca, independent of hetero- or conspecific sperm. This percentage corresponds to that after natural mating. In all cases, spermatozoa in the spermatheca were viable when queens were dissected 3–40 days after insemination. The amount of spermatozoa was below 1 million, except in *A. Koschevnikovi* queens inseminated with conspecific sperm. After insemination of *A. Koschevnikovi* with *A. dorsata* sperm, the percentage of spermatozoa reaching the spermatheca was quite low. But with only two experiments and no reciprocal insemination, these results must be considered preliminary. Woyke (1993) reports that after inseminating *A. Florae* queens instrumentally with *A. mellifera* sperm, some spermatozoa entered the spermatheca, but he did not report the percentage. To a certain extent, a comparable migration of conspecific spermatozoa has been demonstrated in *A. mellifera* queens (Gessner and Ruttner 1977). No reciprocal inseminations were performed. These data suggest that the physiology of the genital duct and its fluid is similar throughout all species. Calculations differ slightly to those of Oldroyd et al. (1998) and Palmer and Oldroyd (2000), but do not alter our conclusions. These findings support the idea that the mode of sperm transfer (sperm injection into the oviducts vs. sperm injection into the spermaduct) influences the filling process of the spermatheca. Injection into the oviducts results in a loss of more than 90 % spermatozoa, whereas with injection into the spermaduct (Koeniger et al. 2000) only about 50 % is rejected. Thus, different sperm numbers together with the different modes of sperm transfer may act as a partial reproductive barrier.

6.24 Postzygotic Barrier

6.24.1 Fertilisation and Hybrids

Ruttner and Maul (1983) collected eggs of cross-inseminated queens of both *A. cerana* and *A. mellifera* 1 h after deposition. In 92 % of the eggs “motile spermatozoa with rapidly undulation movements” could be detected close to the anterior pole. But no larvae developed. Squash preparations from late cleavage stages, fixed and stained, showed normal diploid chromosome sets at the same rates. Serial sections revealed that hybrid eggs of both types could form a ‘pre-blastoderm’, but during the blastula stage the initial cell walls disintegrated again and development ended with a complete breakdown. Apparently there is some variation in the degree of embryonic disintegration.

Reciprocal hybrids with *A. cerana* from Pakistan were all highly disintegrated by the third day of development. Eggs of *A. mellifera* queens fertilised by *A. cerana* spermatozoa, however, showed patterns which at least were similar to the arrangement of an embryo (Ruttner 1988). With these results, complete reproductive isolation was proven between these two species. In 1996, five *A. cerana* queens inseminated with *A. koschevnikovi* sperm produced progeny. Brood counts revealed altogether 338 drone and 454 worker cappings. These queens had 0.6 ± 0.3 million spermatozoa in the spermatheca. In the brood of two queens, we found both drone heads and worker heads. Because of unfavourable circumstances, further analysis was not possible. In 1997, sealed brood combs of two *A. cerana* queens inseminated with *A. koschevnikovi* sperm were kept in the incubator and some hybrids with gynandromorphy characters were reared. From one queen we collected 10 drones and 14 hybrids. In 13 worker-like hybrids, the distance between the complex eyes was more than 1.5 mm; in one bee, this measured only 0.15 mm. All had a long proboscis.

The next step in research on reproductive isolation was the discovery by Koeniger and Wijayagunasekera (1976) that different daily mating periods served as complete mating barriers among sympatric *A. florea*, *A. cerana* and *A. dorsata* in Sri Lanka. These results laid the basis for further developments. In 1988, Ruttner (1988) discussed his research on *A. cerana* and *A. mellifera* and argued “that no premating barrier exists between these two species as is the case between the other species. Because of a not completely finished speciation, it appears totally unjustified to classify this taxon (*A. cerana*) as a subgenus, as was proposed by Skorikov (1929) and Maa (1953). On the contrary, they (*A. mellifera* and *A. cerana*) have to be regarded as being in a late, but not yet finished stage of speciation” (Ruttner 1988, p. 148). So the ‘missing pre-mating barrier’ between species was used as a basis for determination of a taxonomic position. We will postpone commenting on that argument, and first pursue the role of ‘reproductive isolation research’ in the recognition of further ‘new’ *Apis* species.

The rediscovery of *A. koschevnikovi* in Sabah and its recognition as a valid species was based on a combination of different results. The first argument was sympatric distribution with *A. cerana*, then morphometric differences between both species and distinct differences in the morphology of the endophallus were demonstrated (Tingek et al. 1988). After this, evidence for complete reproductive isolation by a separated daily mating period led to the ‘final’ acceptance of *A. koschevnikovi* as a ‘good species’ (Koeniger et al. 1988). Since then, determination of mating periods has become indispensable for the recognition of ‘new’ honeybee species. Research on post-mating barriers between *A. cerana* and *A. koschevnikovi* revealed similarities. In all combinations, 11 hybrids had no stings, and except for three bees which had 1 or 2 drone hind legs, all the others had worker legs. From the other queen we collected 24 offspring: 10 drones and 14 that appeared to be drone-like hybrids, which were recognised because of the dorsal distance between the complex eyes. The distances varied between 0.20 and 1.55 mm; in 11 hybrids, the distance was below 1.0 mm. The ocelli were situated either frontally or between the complex eyes. Six hybrids had short drone and eight had long worker proboscises. All but one had drone legs. In further experiments we will try to produce hybrid queens and test their ability to

reproduce. No data on hybrids between *A. koschevnikovi* queens inseminated with *A. cerana* sperm are yet available. It seems unlikely that the above hybrid bees could form a viable colony. Though we have not yet tried to breed hybrid queens, the results suggest complete reproductive isolation. According to the DNA and morphological results (Arias et al. 1996; Fuchs et al. 1996), *A. cerana* is more related to *A. nuluensis* than to *A. koschevnikovi*. So cross-insemination between these species may result in in 'better' hybrid bees.

6.25 Reproductive Interspecies Isolation of *Apis mellifera* L. and *Apis cerana* Fabr.

One of the Asiatic species, *A. cerana*, is so similar to *A. mellifera* both in morphology and in ethology that it has not always been clear whether these are distinct species. Ruttner and Maul (1983) observed that after instrumental insemination of both *mellifera* and *cerana* queens with heterospecific semen, normal fertilization and cleavage of the eggs were observed. During the blastula stage, however, development stopped and finally ended in a complete breakdown. Even though specific morphological and behavioural characteristics were found later in *A. cerana*, the situation has not been clarified definitively. In northern India, "transitory types" *mellifera/cerana* as well as hybridization were reported (Muttoo 1951a, b; Vats 1953; Sharma 1960). This question still remains unanswered: Is *A. mellifera* completely isolated from *A. cerana* genetically, regardless of the geographic isolation between the two species?

6.25.1 Heterospecific Instrumental Inseminations

The *cerana* queens have been inseminated with *cerana* or with *mellifera* semen since 1966. In spite of the small volume of semen injected (1.0–4.0 μ l) in all cases spermatozoa were stored in the spermatheca of the queen. In a number of cases their quantity was sufficient (up to 1.9 million) for satisfactory fertilization of the eggs laid by the queens. The *cerana* queens inseminated with *cerana* semen produced *cerana* workers and sometimes also drones. If inseminated with *mellifera* semen, only drone brood in a very scattered pattern was produced. The same occurred in the reciprocal cross (*mellifera* queens $\times$ *cerana* drones). In both crosses it was observed that many eggs laid by the queen did not develop and never produced larvae. A *cerana* queen inseminated with mixed semen (*cerana* and *mellifera*) produced only *cerana* workers.

One difference of *cerana* drones compared to *mellifera* drones was that they produced a very small quantity of semen. Moreover, only a small part of the semen from the drones can be collected even if fully mature drones are used. Thus, a high number of drones have to be killed to collect a sufficient amount of semen for an adequate filling of the spermatheca. The studies on insemination in *A. cerana*

were continued by Woyke (1973a). He found that 17 *cerana* drones were necessary (including those which ejaculate incorrectly or not at all) to collect 1 μ l of semen in the syringe. The transfer of *cerana* spermatozoa into the spermatheca is about equally efficient in *cerana* queens as in *mellifera* queens. Twice as many spermatozoa when injected into the oviducts of *cerana* queens were stored in the spermatheca than in the case of *cerana* spermatozoa. Thus heterospecific insemination is as efficient (at least in one direction) as homospecific.

6.25.2 Development of the Zygote

The embryonic development of reciprocal crosses between *Apis mellifera* and *Apis cerana* was studied morphologically by means of serial sections. During the first 24 h of development normal superficial cleavage led to the formation of the so-called “pre-blastoderm”—a layer of low cells all over the egg surface with the residual “secondary periplasm” underneath. The next step of blastodermal differentiation, the formation of cylindrical cells in the embryonic areas, was never performed by hybrid eggs of both types. Instead, the initial cell walls started to disintegrate again and the nuclei appeared to migrate back into the secondary periplasm. This disintegration proceeded from polar and dorsolateral areas, more or less to the ventral area. Not affected by this process was a special group of cells along the dorsal midline which corresponds to the extra-embryonic amnion.

Evidently, in the detailed study of the consecutive steps in the process of sexual reproduction in the two species of honey bees, *A. cerana* and *A. mellifera*, an astonishing degree of congruence was found. This applied even to the rather complex behavioural and physiological situations, as the orientation during the mating flight or the storage of spermatozoa in the spermatheca. Two effective blocks to hybridization exist, block no. 1 which occurs during the copula and block no. 2 during the zygotic development after successful fertilization of the egg. Block no. 1 is the consequence of important morphological differences in the copulatory organs of the drones. The chitinous plates with their sharp edges of the *mellifera* endophallus seem to hurt *cerana* queens severely, at least in some cases. Block no. 2 makes it impossible to determine whether *cerana* semen is transferred to *mellifera* queens and how frequently this event occurs. It is astonishing that *mellifera* spermatozoa instrumentally injected in the oviducts of *cerana* queens or vice versa are transferred into the spermatheca, survive there for a yet undetermined period, and migrate through the narrow ductus spermaticus to finally fertilize the heterospecific eggs. This is exactly the same with homospecific spermatozoa, even to the fusion of the sperm nucleus with the pronucleus of the egg and the start of the development of a hybrid embryo. The second block (abnormal development), as observed in our hybridization experiments, started with the formation of the blastoderm. Although there was apparently some variation in the degree of disturbance of the embryonic development after cleavage, the basic phenomenon was identical in both reciprocal hybrids. These findings are congruent with the results of species hybridization experiments in many other animals where

the development proceeds normally to the blastula stage (which corresponds to the blastoderm of insect eggs), but is blocked or disturbed from the gastrula stage onward (review by Stebbins 1958). Such early findings have supported the hypothesis that cleavage is controlled by the cytoplasmic system of the egg rather than by the genome (Duspiva 1969). The development up to the blastula or a corresponding stage is controlled by “maternal messengers”. Differential gene expression, followed by differential protein synthesis, usually does not start earlier than at blastula-like stages (Duspiva 1969; Davidson 1977; Ede 1981). Morphologically similar anomalies of embryonic development are sometimes observed in the honeybee as a result of inbreeding. However, the defective development of inbred embryos is highly variable, affecting sometimes also the earliest steps of development. These developmental blocks are understood as maternal effects. The inbred female is unable to produce the proper ooplasmic system during oogenesis. On the basis of differences in morphology and behaviour between *A. mellifera* and *A. cerana*, two more arguments can be added to support the fact that they are indeed separate species.

The mating flight times of native *Apis cerana japonica* and introduced *A. mellifera* were compared in the same bio-type in Japan. The queen flight times for *A. cerana japonica* and *A. mellifera* were 1315–1700 h, 1215–1500 h, and those of the drones were 1315–1630 h, 1130–1500 h, respectively. Both the hive departure and mating flight times of *A. mellifera* were 1.5–2 h earlier than those of *A. cerana japonica*. Successful mating flights of queens occurred between 1300 and 1440 h in *A. mellifera* and between 1445 and 1635 h in *A. cerana japonica*.

6.26 Artificial Insemination

Now that inter-specific mating can actually happen under natural conditions, one is prompted to pose two questions (1) what happens after such matings occur? and (2) is there any hybrid offspring produced? None of the eggs hatch because of post-zygotic barriers between the species. Artificial insemination between *A. cerana* and *A. mellifera* has been applied by researchers (Ruttner 1969; Ruttner and Maul 1983; Woyke 1973a, b; Koeniger et al. 1996b; Koeniger and Koeniger 2000; Phiancharoen et al. 2004), but no hybrids have been obtained thus far. Ruttner (1988), described the detailed developmental process in eggs laid by the queen after hetero-specific instrumental insemination. The hetero-specific spermatozoa can enter the spermatheca, are able to survive there, and can fertilize eggs. 24 h after fertilization, cleavage is observed to the blastula stage of the zygote. Then, however, the cell walls start to disintegrate and nuclei migrate into the secondary periplasm to accumulate in the antero-ventral part of the zygote and then to degenerate completely later on. Thus, no hybrid larva or imago ever develops.

Yoshida (1994), used the mixed semen of *A. cerana* and *A. mellifera* drones to inseminate *A. mellifera* virgin queens. By using different mixed ratios of the two specific spermatozoa (approximate spermatozoa concentration ratio of 3 mm³ of *A. mellifera* semen + 1 mm³ *A. cerana* semen, 9:2, 2 mm³ of *A. mellifera* semen

+ 2 mm³ of *A. cerana* semen (6:4), 1 mm³ of *A. mellifera* semen + 3 mm³ of *A. cerana* semen (3:6) was 79.5, 53.6 and 26.5 %, respectively), he found the hatchability after the queen laid eggs produced only *A. mellifera* workers, inter-specific fertilization resulted in non-viable larvae. Koeniger (1996), reported inter-specific hybrids between *A. cerana* and *A. koschevnikovi* produced by artificial insemination have low fertility and the hybrid colonies are probably non-viable. Phiancharoen et al. (2004) used spermatozoa from drones of four species (*A. mellifera*, *A. cerana*, *A. florea* and *A. dorsata*) to respectively inseminate *A. mellifera* queens. They studied survival rate of each specific sperm type and the rate of eggs fertilized by each specific spermatozoon. The results showed that nearly 100 % of *A. cerana* and *A. mellifera* spermatozoa were still alive 4 weeks after insemination, but the motility of *A. florea* and *A. dorsata* spermatozoa decreased to a large extent, 83.4 and 61.2 % respectively, after 3 days and only a small proportion were still alive in the queens' spermathecae. As for fertilization rate, 57 % of *A. mellifera* eggs were fertilized by *A. mellifera* spermatozoa, 40 % eggs fertilized by *A. cerana* and *A. florea* spermatozoa, while less than 20 % by *A. dorsata* spermatozoa. The fluid in the queen's spermatheca played an important role in the survival rate and fertilization success rate of the hetero-specific spermatozoa, but no inter-specific hybrid offspring emerged.

6.27 Instrumental Insemination in *Apis cerana*

Ruttner et al. (1973) reported that morphology of the vagina of *Apis cerana* queen, in particular the open valve-fold makes instrumental insemination easier than in *Apis mellifera* in the drone, both the quantity of semen and the concentration of spermatozoa in it are lower than in *Apis mellifera*. In the seminal vesicles about 1.5 million spermatozoa were found per drone and in the ejaculated fluid about 1.0 million per drone, they further concluded that to ensure full insemination, *A. cerana* queens must mate with more drones than is necessary for *A. mellifera* queens.

Apis cerana indica drones produce produce eight times lower volume of semen (0.20 mm³) than *A. mellifera* drones (1.60 mm³). The concentration of semen is 62 % (4.655 million/mm³) of that found in *Apis mellifera* (11 million) (Woyke 1960). The penetration ability of *Apis cerana indica* spermatozoa into spermatheca is also lower than that of *Apis mellifera*. Naturally mated *A. cerana indica* queens had lower number of spermatozoa in the spermatheca (the highest number found: 2.665 million) than *A. mellifera*. Nevertheless, the queens of *A. cerana indica* must mate with higher number of drones (15 in 1 flight or 30 in 2) than do the *Apis mellifera* queens (average 8 mating in 1 flight). It is possible to collect sufficient amount of *A. cerana indica* semen for artificial insemination. The injection of semen into oviducts of *A. cerana indica* is not more difficult than in *A. mellifera*. But the number of drones used for insemination must be higher than in *Apis mellifera*. Successful artificial insemination with one drone is possible in *Apis mellifera* but these results in only few of the spermatozoa entering the spermatheca of *A. c indica* queens. Insemination with 1–4 mm³ of semen resulted in 0.411–1.21 million of spermatozoa in the spermatheca.

Queens inseminated with doses higher than 3 mm^3 of semen produced at the end of the season exclusively worker brood. Two or three inseminations with 3 mm^3 of semen each are recommended. To do this semen from 40 to 60 drones must be collected for which 100–150 of them must be killed.

6.28 Instrumental Insemination

Instrumental insemination of honeybee queen is one of the advanced technologies and an essential tool for ensuring the selective mating for stock improvement. A brief account of development of instrumental insemination and techniques for collection and storage of semen, insemination process and post-insemination care of the queen bee have been described. Need for the artificial/controlled insemination in honeybees arose for controlling the parentage for stock improvement. Instrumental insemination is an essential tool for the research scientists requiring specific crosses and for the beekeepers involved in a bee breeding programme. Mating control in queen honeybee could be made possible only as per given:

1. Development of natural methods for controlled mating in isolated mating yards and
2. Development of artificial insemination technology in honeybees through hand mating and subsequently through instrumental insemination.

6.28.1 *History of Controlled Insemination in Honey Bees*

6.28.1.1 Hand Mating

Earliest attempt was made by McLain (1887) who placed drops of semen upon vulva of a queen bee. Late efforts were made by many scientists (1917–1933) to introduce the copulatory organs of drone into the queen's sting chamber but only Quin (1923) with the help of laid law claimed some success. The most extensive studies on manual mating were conducted by Muzalesvskii and Kozlov (1933). They used ready-to-mate queens and claimed 10–15 % success. Subsequently, during 1950–1956, several persons experimented using different types of apparatus. However, Tryasko (1956), using modified method of hand mating, showed that only traces of sperms (less than 2 % of the normal filling) were found in the spermatheca. Normal brood rearing was observed only in those cases where queens mated naturally afterwards without being noticed by the apiarists. All queens, that were not allowed to mate subsequently, produced only the drone brood.

First attempt to inseminate the queen bee using instrumental insemination equipment was made by Huber (1738–1791) by introducing the drone semen into the vagina of the queen bee by means of a hair-brush pencil. Many other workers tried to use a syringe but without any success. The first successful attempt in instrumental

insemination was that of Watson (1927) who used a micro-syringe clamped in a micro-manipulator. The queen was tied to a wooden block using several silken threads. The sting was opened by a pair of forceps held in hand. Later Nolan (1932, 1937) devised a simple apparatus for artificial insemination to introduce more semen into the vagina of the queen. Laidlaw (1944) discovered the role of the valve-fold and reported that for successful insemination, the semen must be injected beyond the valve-fold in the median oviduct. Laidlaw (1944) modified the apparatus by controlling the movements of needles and syringes with screws. Mackensen and Robert (1948) modified the apparatus of Nolan (1937) and obtained better results over those of latter.

6.28.1.2 Development in Stimulating Egg-Laying by Inseminated Queen Bee

Laidlaw (1930) studied the utility of CO anesthesia to queen bee. Mackenson (1943–1947) discovered that two doses of anesthesia for queen bee with CO would stimulate the queen to initiate oviposition.

6.28.1.3 Development in Semen Storage Studies

Taber and Blum (1960) successfully demonstrated the storage of semen in vitro at temperatures above freezing point for longer timetable (1961) and successfully shipped the semen between countries and continents. Poole and Taber (1970) successfully stored semen at 13–15 °C for 35 weeks after mixing it with streptomycin and keeping it in flame-sealed glass capillary tubes. Kumar and Gupta (2003) found that in semen stored at room temperature (23–32 °C) in glass capillaries the sperm motility was 46 % after 60 days of storage. Sawada and Chang (1964) reported the semen resists very low temperature but Lensky and Schinde (1967) reported that semen degenerates fast at temperatures below the freezing point. Ruttner et al. (1972) and Woyke (1973a, b) demonstrated that *Apis cerana* Fab. queens may be inseminated by the same method as *Apis mellifera* Linn.

6.29 Instrumental Insemination Equipment

Good results in large-scale use of instrumental insemination depend upon the simplicity and reliability of the insemination equipment. The standard model of insemination apparatus consists of a heavy base supporting the two hook-holding blocks with ball bearings, syringe-holder and a queen-holder. It is a specially designed manipulator, which facilitates both collection of drone semen and insertion of semen into the vaginal orifice of the queen. Semen is collected in the syringe; the assembly of which is shown. The parts of the instruments which have contact with the semen and saline

solution must be sterilized to avoid problems with contamination. A house-hold pressure cooker is useful to sterilize the metal parts of the instruments. Plastic/rubber parts may be sterilized using alcohol.

6.30 Management of Queens and Drone Bees for Instrumental Insemination

6.30.1 (I) Management of Queens Bees

6.30.1.1 Rearing and Maintaining Queens

In establishments where instrumental insemination (II) is practiced, an efficient and productive method of mass queen bee production must be adopted. Large and vigorous queens make the instrumental insemination procedure easier and only good queens justify the extra effort involved. Studies on mass queen rearing conducted at PAU, Ludhiana has revealed the use of artificial queen cell cups of 9.0 mm diameter and young worker larvae (< 24 h) from the selected breeder colony for grafting and efficient rearing from older larvae (Gatoria et al. 2004a). Double grafting is often considered to produce large queens. The number of open queen cells in the cell finishing colony is an important factor in queen quality and it should never exceed 30. Queen bees can also be reared using queen cup kit apparatus (Gatoria et al. 2004b).

6.30.1.2 Emergence and Marking the Queen

Sealed queen cells can be given individually to queen-less nuclei but placing the sealed queen cells in an incubator at 35 °C about 2 days before emergence should be preferred. A few hours after emergence, the queens are marked as per the international colour codes using coloured plastic disks or making colours white, gray, yellow, red, green and blue. Clipping the distal part of one of the fore wings, i.e., left wing or right wing in alternate years, is also helpful for determining age of the queen bee.

6.30.1.3 Maintaining the Queens after Emergence

Push-in cage method for queen introduction is considered to be satisfactory. Maintaining queens in small cages is very laborious. Under favourable conditions of climate and nectar sources the storage for virgin for a short duration and instrumentally inseminated queens (for longer duration) is possible in special queen-less nursery colonies termed as queen banks. According to the studies conducted at PAU, Ludhiana up to 20 mated and laying queen bees could be kept and maintained in a strong dequeened queen bee bank colony, for over 4 months by frequent addition of ready

to emerge brood from other colonies (Singh and Gatoria 2003). To prevent uncontrolled mating, the virgin queens must be confined to the mating nuclei using a large piece of queen excluder. Caged queen must be inserted in the middle of the cluster between two combs. Entrance of the nuclei should be large enough so that it must not be clogged by the drone bees attempting to fly out. Virgin queens left free in nuclei should be inseminated at 5–6 days' age.

6.30.1.4 Preparing Queens for Insemination

If a queen is older than 5 days and good flight weather prevails, she must be caught up before 1000 h or after 1600 h because if it tried to catch it between 1000–1600 h, she may try to escape for natural mating. After catching, queen should be placed in small cage with 5–10 attendant bees and left in the nucleus until insemination. If several queens are to be inseminated one after the other, they should be fed and provided with several worker bees and kept in a cabine at about 25 °C. Caged queens, just before insemination, can be permitted to fly against a window pane for short time causing defecation.

6.30.2 (II) Management of Drones

6.30.2.1 Rearing and Maintenance of Drones

Availability of sexually mature drones for instrumental insemination is also very important task. During early build up period. Simply place an empty drone comb in the brood nest of the selected colony for lying of drone eggs. Alternatively, queen bee can be confined to drone comb in an excluder cage or by using vertical queen excluder. Older drone combs in which several generations have been reared should not be used again and again. Drone rearing colony should have higher bee strength, abundant food stores, good population of nurse bees, and young worker brood on both sides of the drone brood comb.

6.30.2.2 Obtaining Drones from Worker Bees

When genotype of worker bees is to be exploited, about 500 young worker bees are obtained by shaking in a receptacle. A medium sized nucleus is formed on drone combs and its is fed profusely. The nucleus is then placed in an isolated location. After few days, the colony will become full of laying workers. The comb with drone eggs laid, but without bees is then transferred to the middle of the brood nest of a queen right nurse colony, with the queen being prevented to reach that comb (using queen excluder cage) and lay eggs. The drones thus produced from this comb will conform to the genotype of laying workers.

6.30.2.3 Maintenance of Adult Drones

Drones required for semen collection must be in an apiary near to the insemination laboratory. For maintenance of these drones, following points must be considered.

1. Drone comb is placed in an incubator 2 days before emergence. At 2–3 day interval, the emerged drones are marked and released in a strong colony. Change the colony every 3 days to recognize the age of marked drone. These drones are then placed in a colony, which does not have its own drones. A drone rearing colony should not have more than 2,000 drones. Making the drone nurse colony queen-less will ensure better nourishment of the drones. In this colony ensuring free flight, continuous light (by providing an overhead drone flight cage) and simulative feeding is advantageous.
2. Alternatively, the comb of the drone brood for emergence should be given to a colony or a strong nucleus made up on empty combs with shook swarm method over a queen excluder to remove its own drone bees if any. A mated queen several days before the emergence of the drones is also given. Gate of the hive is also provided with a piece of queen excluder to check the entry of alien drones.
3. If required, hold the drones with nurse bees outside the hive (in drone cages) from emergence to sexual maturity. The temperature must be more than 30 °C, else at low temperature, the migration of spermatozoa to seminal vesicles will be retarded. Ensure proper temperature or migration of spermatozoa to seminal vesicles will be retarded. To ensure proper temperature, drone cages with drones can also be placed inside the colony in middle of the brood nest.
4. Store drones after emergence till utilization in drone cages/excluder cages in the colony itself. Each cage may hold drones of one different origin marked with different colour. Supplementing the young brood from other colonies around the drone cages and continuous feedings to the drones nursing colony are necessary for better nourishment of the drones. Thus, groups of drones of different genotypes can be kept in different cages in the same drone nurse colony.
5. If the drones caught have no chance to fly in the drone nursing colony, they can be permitted to fly in a drone flight cage measuring 50 × 40 × 40 for 10 min before semen collection. During this time the drones will defecate and after flight activity, they will be much better and the risk of contamination of semen is also reduced.
6. For rearing drones off-season, create artificial swarming conditions by reducing the space and number of combs, liberal addition of bees from other colony, supplementary pollen and sugar feeding are also necessary. After egg laying, dequeen the colony for ensuring better care of the drone brood under emergency impulse.
7. Two CO₂ treatments/anesthesia for 15–20 min (once 5–6 days after queen emergence, and again 2–3 days later) may stimulate the virgin queen to lay unfertilized eggs. Such drown layer queens be maintained on drone combs in strong colonies, as the drones reared in worker cells are smaller in size, producing less semen.

Effect of the Flight Activity on the Sexual Maturity of Drones

The beginning of flight 7–10 days after emergence is not proof of sexual maturity of drones. Migration of mature spermatozoa into the seminal vesicle starts 6–9 days after emergence. Maximum aversion ability in drones is reached after 12th day of emergence. Thus to get mature drones for semen collection, drone rearing must be initiated at least 20 days prior to queen rearing. Reports regarding stimulatory effect of flight and on ability to drone eversion are contradictory. But Mackensen (1955) found 10 % increase in usable semen from free-flying drones as compared to caged ones.

Length of Life and Performance of Drones

Drone longevity depends upon rearing, nursing and seasonal weather conditions. Drone longevity has been recorded unto 21–70 days but proportion of long-lived drones has been reported to be only 3–5 %. Caging shortens the life of the drones. Eversion ability in old drones is also found between 76–95 %.

6.31 The Insemination Technique

6.31.1 Requirements

In addition to queen and drones, insemination instruments, complete with a syringe, hooks accessories and a source of carbon dioxide for anaesthetization are required. A stereo microscope with a suitable cold light source is also essential. The detailed list of the materials required is given in (Table 6.1):

6.31.2 Filling of the Syringe

The syringe is sterilized and filled with sterile saline solution. A common physiological saline solution (0.9 % sodium chloride solution) can be used. Sterile water can be obtained in a glass ampoule from a scientific supply company or a pharmacy. Alternatively, distilled water run through a bacteria filter with a pore size of 0.2 mm can be used. An antibiotic is also added to prevent contamination/infection.

6.31.3 Attaching the Glass Tip to the Syringe

The blunt end of the glass tip is pushed into the syringe end/sleeve and the tip is attached with the sealing tube for squeeze-sealing. The functioning of the hydraulic system of the syringe can be easily tested with the movement of control knob

Table 6.1 List of tools, chemicals and other material required

1	Disinfectant (sterilium/alcohol)
2	Chloroform
3	Boar sperm diluent of normal saline
4	Distilled water with 80–120 mm clearance
5	Towel
6	Cleaning paper
7	2 and 20 ml injection syringes
8	Cleaning tissue
9	Sterilized ear buds
10	Camel hair brush
11	Two petri dishes
12	Two 500 ml beakers
13	Autoclave
14	CO ₂ cartridge/cylinder with regulator
15	Incubator
16	A pair of scissors
17	Forceps
18	Stereo microscope (15–20 ×)
19	Cold light source
20	Insemination apparatus
21	Queen marking discs/pens/colours
22	Queen cage candy
23	Drone cages
24	Drone flight chamber
25	Mature drone bees (15–20 days old)
26	Ready to mate queen bees (5–7 days old)
27	Queen introduction cages
28	Mating nuclei

6.31.4 Stereo Microscope

Magnification required for insemination is 15–20 × and a working space of 80–120 mm below the objective is required. Not all types of microscopes are suitable to use with the insemination instrument. Hence, be sure that the microscope and instrument chosen must be compatible. A cold light source, which does not produce heat, facilitating proper illumination is necessary for insemination operation.

6.31.5 Eversion of Endophallus of Drone

The reproductive organs of the drone bee are shown in the Fig. 6.1.

To collect semen, already marked mature and healthy drones are used. Before actual collection of semen the drones are stimulated to evert their bulb of endophallus (penis) inside out so as to expose the semen. Drone eversion is obtained in two steps, i.e., partial eversion and finally complete eversion to expose the semen on the bed of thick mucus on the surface of bulb of endophallus.

The drone is stimulated by decapitation, which results in partial or full eversion. Alternatively, partial eversion is obtained by subjecting the mature drone, caught from drone flight cage, to some chloroform fumes. Further, for full eversion, grasp the drone laterally from anterior part of abdomen and slightly press it to obtain semen. Semen is light pinkish to cream-coloured, appearing mottled or cloudy, on a thick white bed below.

6.31.6 *Semen Collection*

A small air space in the syringe is created by drawing some amount of saline, using plunger of syringe. This will prevent dilution of semen with saline. The ejaculated drone (fully everted bulb of penis/end phallus) is brought near the syringe tip under the microscope. After making contact with semen, it is skimmed out from the more viscous mucus layer below. Avoid placing the tip of the syringe deep into the thick mucus layer. Also avoid drying of the semen at the tip by collecting a small amount of saline in the tip of the syringe between drones.

Repeat this process to collect the quantity of semen needed. Small amount of saline solution is drawn into the syringe every time when you stop semen collection to prevent sealing/clogging-up of the tip by dried semen. Expel this saline solution out when you again start semen collection. Each queen is required to be given 8 μ l of semen (i.e., equivalent to 12.5 mm length of glass capillary). An experienced person can collect this much semen in just 5 min.

6.31.7 *Placing the Queen in the Holder*

Place the queen into the cylindrical backup having a small hole on one end. When queen reaches the end of the tube with the small hole, she will backup into the holding tube which is immediately held next to this backup tube. The queen moving backward will protrude its abdomen out of the tapering end of the holding tube. The queen can be helped in this process by blowing lightly with mouth from the other end. The holding tube with the queen is then put back on the queen-holder of the instrument, which is connected to the carbon dioxide line. Flow of CO₂ is kept constant at 1 bubble/s. The tube is kept in such a manner that the back of the queen is on the right side. The queen is ready for insemination when she becomes motionless.

6.31.8 *Anaesthetization*

Carbon dioxide through the tube connected with the queen-holder is used to keep the queen anesthetized continuously during the insemination. A slow and steady rate of

carbon dioxide bubbled through water, adjusted at the rate of one bubble per second is generally used to keep the queen quiet during the process. The carbon dioxide treatment is also important to stimulate egg-laying. Two treatments are necessary to initiate egg-laying, one during the insemination procedure and a second, one day before or one day after the insemination. Longer exposures should be avoided as this can shorten life expectancy of the queen bee.

6.31.9 *Opening the Sting Chamber*

The reproductive organs of queen bee showing the position of valve-fold. For injection of semen, the inseminating syringe must pass through the vaginal orifice and vaginal passage beyond the valve-fold. As the anesthetized queen in the queen-holder becomes quiet, sting chamber of the queen is opened with holding hooks. Ventral hook, by keeping to the left (ventral side of the queen, is first put in the ovipositor to stretch the ventral side (last sternite) of the abdominal tip. The sting hook which is holed, is used to thread the sting by keeping it to the right (dorsal) side of the queen bee. When the hooks are properly positioned, the tissue will stretch to form a large triangle. Within the triangle is a 'v'-shaped wrinkled tissue defining the vaginal orifice. Below this is the location of the valve-fold, which is not readily visible.

6.31.10 *Injecting the Semen*

The whole process of opening of sting chamber of a queen bee and injection of semen is done under a microscope. The syringe tip having semen is passed just above and slightly right to the 'v'-shaped wrinkled tissue, i.e., the vaginal orifice'. To bypass the valve-fold, a slight zigzag motion of the syringe tip is used. This is essential to move the valve-fold for the passage of the semen. If the syringe is properly placed, there will not be any movement of surrounding tissues when it is inserted further, in the vaginal orifice.

The semen can now be injected and it should not leak out if the surrounding tissue moves with the syringe tip, you have not bypassed the valve-fold and the semen would leak out/back-up.

6.31.11 *Post-Insemination Care of the Queen Bee*

Inseminated queen bee can be dropped between the combs while it is still anaesthetised. But it is better to put it into the queen cage with candy and let the queen after insemination become active in the incubator. Upon activation, introduce the queen in the queen-less colony or nucleus. The tendency of the queen for mating flight persists even after one insemination or one anaesthesia with CO₂. Therefore, queen must remain confined in the colony until second insemination or anaesthesia is completed. Queen guard should not be removed from the entrance until queen

starts oviposition. The first 24 h after insemination is a critical period. Caged queens must always be near the brood nest because at lower temperature, transfer of injected spermatozoa to spermatheca, occurs. Hence, newly inseminated queens should be kept at brood nest temperature and be attended by nurse bees to facilitate sperm migration. Sperm migration is influenced by environmental conditions. Therefore, queens should not be subjected to cold or isolation. Queen bee inseminated with less than 5 mm³ semen can be maintained under the normal colony conditions. But queen bee inseminated with semen from single drone should be kept in nuclei where a lack of room restricts egg-laying and thereby prolongs considerably the life and should be fertilised, maintained in a strong queen bee bank/reservoir colony (Singh and Gatoria 2003), for over 4 weeks.

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Chapter 7

Pheromones

7.1 Introduction

An important area of physiology of the honeybee (*Apis mellifera*) is chemical communication between individuals and castes in the swarm, which maintains its integrity and function. The highly complex social organisation of honeybees is mediated through pheromones. Releaser pheromones cause rapid changes in the behaviour of the recipient, whereas primer pheromones have relatively slow and long-term effects on the physiology and behaviour of the recipient. Queen retinue pheromone (QRP) is a blend of the nine compounds (9-oxo-(E)-2-decenoic acid, (R)- and (S)-9-hydroxy-(E)-2-decenoic acid, methyl *p*-hydroxybenzoate (HOB), 4-hydroxy-3-methoxyphenylethanol (HVA), methyl oleate, coniferyl alcohol, palmityl alcohol, and linolenic acid) and acts as a releaser pheromone by attracting worker bees to the queen. QRP also acts as a primer pheromone by physiologically inhibiting the ovary development of worker bees. An essential component of QRP, 9-oxo-(E)-2-decenoic acid, acts as a long-distance sex pheromone. Defensive behaviour of honeybees is induced and modulated by alarm pheromones. The essential alarm pheromone component is isopentyl acetate (also known as isoamyl acetate, or IPA). The unsaturated derivative of IPA, 3-methyl-2-buten-1-yl acetate (3M2BA), was found in colonies of Africanised honeybees. The Nasanov gland of worker bees produces a pheromone (a blend of nerol, geraniol, (E)- and (Z)-citral, nerolic acid, geranic acid and (E, E)-farnesol) that acts as an attracting signal. This pheromone is used for aggregation (during swarming). Adult worker bees also produce a substance, ethyl oleate (EO), which has a priming effect. EO is produced by adult forager bees and acts as a chemical inhibitory factor to delay age at onset of foraging (the presence of older worker bees causes a delayed onset of foraging in younger individuals). Chemical cues on the surface of larvae called a brood pheromone (BP; ethyl and methyl esters of palmitic, linoleic, linolenic, stearic, and oleic acids, E- β -ocimene) are important in the communication between brood and worker bees. This pheromone modulates the feeding behaviour of worker bees, inhibits the activation of the worker ovary, induces worker bees to cap brood cells, increases the activity of the hypopharyngeal glands of nurse bees and modulates the behavioural maturation of worker bees.

Communication among insects is extremely important for their survival, especially for social insects that live in complex colonies. Honeybees are well known for their chemical communication and use of olfactory cues (Free 1987; Free et al. 1983; Leal 2010). For example, honeybees can navigate to food sources by detecting floral scents and by orienting towards food-marking pheromones or, possibly, cuticular hydrocarbons (cues) deposited as footprints (Balderrama et al. 1996). Honeybees smell or detect pheromone with their antennae using odorant-binding proteins in sensillum lymph. They produce volatile and non-volatile chemicals as signal molecules from their exocrine glands to communicate with others of the same species or with other species (Kaissling 1977). These signalling chemicals are often called semiochemicals (Kaissling 1972).

The secretory product of worker mandibular glands consists of 10- and 8-carbon acids that have an oily appearance. In young workers, this gland produces a lipid-rich white substance that is mixed with the secretion of the hypopharyngeal glands to make royal and worker jelly that is fed to the queen or workers. In old workers (foragers), the gland also produces 2-heptanone (2-HPT), a volatile substance that accumulates in the central reservoir, the amount of which progressively increases with increasing age (Engels et al. 1997). This compound can repel guard bees, and is a potential component of alarm pheromone. On guards, 2-HPT has been reported to have either an attractive or a repulsive effect, according to the season. A foraging bee may mark a nectar-depleted flower with 2-HPT (Balderrama et al. 1996; Boch and Shearer 1962, 1971; Blum 1969, 1982, 1992; Blum et al. 1978; Crewe and Hastings 1976; Engels et al. 1997; Guirfa 1991; Suwannapong 2000). In foraging bees, 2-HPT can have a temporary, repulsive effect on the visitation of flowers. Suggesting that, it is “forage marking” pheromone (Vallet et al. 1991). Mandibular glands of Thai honeybees mainly produce (Z)-11-eicosanol instead of 2-HPT (Suwannapong 2000; Suwannapong et al. 2010a).

Maschwitz (1964) suggested that mandibular glands produce alerting pheromones (in Blum 1969). Shearer and Boch (1965) identified 2-HPT from mandibular gland secretions. When filter paper with 2-HPT was placed at the hive entrance, guard bees were alerted and attacked the paper. This is consistent with suggestions by Boch and Shearer (1962) that 2-HPT has two functions: as an alarm pheromone and as a foraging repellent pheromone. However, it is possible that cuticular hydrocarbons deposited by bees walking on a depleted food source are sufficient to indicate that food sources are depleted. It is possible that 2-HPT is a repellent at high concentrations and an attractant at low concentrations (Boch and Shearer 1971; Kerr et al. 1974; Vallet et al. 1991). The conflicting hypotheses of Guntima Suwannapong Mark Eric Benbow 42 and James C. Nieh 2-HPT’s function on a depleted resource (repellent or attractant) require additional study. In *A. florea* and *A. cerana*, the response of guards towards 2-HPT was different from the response of foraging bees (Suwannapong et al. 2010a, 2011).

The response of antennal sensillum of *A. florea* to low concentrations of 0.1 % 2-HPT was higher than the response to higher concentrations of 5 and 10 % 2-HPT (Suwannapong et al. 2011). In contrast, *A. cerana* foragers exhibited a stronger response to a 10 % 2-HPT compared with that of 0.1 and 1.0 %. The membrane

potential of foragers following exposure to 2-HPT was higher than that of guards in both *A. florea* and *A. cerana* (Suwannapong et al. 2010c, 2011). One of the components of sting pheromone, IPA, releases strong alarm behaviour in bees. Aggressive behaviour can be observed when mandibular glands or crushed heads of worker bees are presented at the hive entrance (Shearer and Bosch 1962, 1965). IPA is 20–70 times more effective as an alarm pheromone than 2-HPT (Boch and Shearer 1971; Boch et al. 1975).

All species of *Apis* have alarm pheromones and the compounds are generally similar among honeybee species with the exception of *A. laboriosa*, the giant Himalayan honeybee (Vander Meer et al. 1998; Harborne 1993). Africanised bees secrete alarm pheromones with the same concentration of IPA as other bee species, but with more 2-nonanol and decyl acetate. These differences may lead to a more aggressive response to alarm pheromone. Honeybee species that have open nests such as *A. florea*, *A. andreniformis*, and *A. dorsata* tend to have alarm pheromones that persist longer such as 2-decenyl acetate (Vander Meer et al. 1998). Workers of Thai *Apis* species also have these compounds, along with 9-HDA and ODA, which are normally not present in *A. mellifera* worker glands. Queens and workers of each different *Apis* species have different combinations of mandibular compounds (Plettner et al. 1996). The sting glands of *A. dorsata* and *A. florea* have an additional pheromone besides IPA, 2-decen-1-yl-acetate (2-DA). Upon presenting this pure compound, these Thai species exhibited a prolonged alarm reaction as compared with the reaction for pure IPA. A mixture of IPA and 2-DA had a similar effect on the behaviour and reaction time, as did sting extracts (Koeniger et al. 1979).

“Social insects are a rich source of volatile compounds” (Blum and Brand 1972). Among these volatiles are pheromones, chemicals emitted by the individuals of a species to communicate messages to other individuals of the same species. Pheromones are chemical messengers, secreted by one honeybee that elicits a behavioural or physiological response by another honeybee. It is produced as a liquid and transmitted by direct contact as a liquid or as a gas. Honeybees use pheromones to communicate with each other in almost the same way we humans use our voice. This communication is primarily used for the interactions of members of the same colony; and it is thought that the colony is regulated chemically by the pheromones produced by the queen. We now know that worker bees and drones produce as well as respond to pheromones, and, that the queen produces and uses pheromones considerably more than the other two castes. Pheromones are not single chemicals, but rather a complex mixture of numerous chemicals in various different percentages of the total. Further, one single pheromone may have multiple functions, and, in contrast, a single behavioural response probably involves more than one pheromone. However, technology has had almost exponential growth over the last 50 years, and the chemists of the next century will identify these now unknown chemicals and even synthesise them. Pheromones must be divided into two different classifications: releaser pheromones that trigger an almost immediate behavioural response from the receiving bee; and the primer pheromones, which cause the receiver bee to exhibit an altered behavioural response at some future time. Although this division of releaser and primer pheromones is our current understanding of their actions, it is important

Table 7.1 Components of Nasonov gland secretions and their concentration. (Pickett et al. 1980)

Extracting methods	Pheromones detected	Microgram per bee	
		<i>A. cerana</i>	<i>A. mellifera</i>
Syringe extraction	Citral	Not quantified	—
	(Z)-citral	Not quantified	—
	Geraniol	Not quantified	—
Abdomen dipping	(E)-citral	20.0	—
Dispersing excised	(E)-citral	3.8	—
	Geraniol	2.5	—

to realise that a single pheromone can function as both a releaser and primer under certain specific conditions.

7.2 Functional Role

In broad generalities, pheromones affect many things such as mating, swarming, alarm behaviour, security, social togetherness, sexual attractant, inhibition of queen rearing and ovarian development in workers; the glue that holds a large population of bees together as a single functioning unit is a “homing” message to other colony members, queen supersedure caused by loss of pheromone, inhibition of queen cell development on the “face” of the comb, and numerous other actions. Perhaps, to most of the beekeepers, the two most important pheromones of all the chemicals produced in the mandibular glands of the queen are 9-oxo-2-decenoic acid (9-ODA) and 9-hydroxy-2-decenoic acid (9-HDA). 9-ODA not only inhibits queen rearing as well as ovarian development in worker bees, but is also a strong sexual attractant for drones when on a nuptial flight. It is critical to worker recognition of the presence of a queen in the hive. 9-HDA promotes stability of a swarm, or a “calming” influence to the natural excitement of a swarm.

These two chemicals, 9-ODA and 9-HDA, are often referred to as “queen substance”. We know that this combination perform critical functions of worker retinue formation and development of colony cohesion. It is this combination that acts as the “glue” that binds all the elements of colony segments, even very large populations, into a single functioning colony unit. Often overlooked, is the “footprint pheromone”, the oily secretion of the queen’s tarsal glands that is deposited on the comb as the queen walks across them. This pheromone, which also diminishes as the queen ages, inhibits queen cell construction (thereby inhibiting swarming).

Many beekeepers are familiar with the Nasonov (Nassanoff) gland pheromone, and often remark about seeing bees “scenting”. This gland produces at least 7 different terpenoids, the most abundant being geraniol and citral (Table 7.1). These are easily and cheaply synthesised and are used as swarm attractants or “homing” pheromones to a swarm trap. In normal use, the worker bees spread the Nasonov scent when foraging for water to aid other water foragers to the site, “scenting” at the doorstep

of the colony to guide colony bees home, and scenting at a new site during a swarming procedure to notify other swarm members.

All beekeepers are quite familiar with the sting alarm, although many are not aware that this is a pheromonal action. The koschevnikov gland, near the sting shaft, produces an alarm pheromone consisting of more than 40 chemical compounds, of which IPA is noteworthy, which signals to other workers to aid in the attack and plant another sting close to the impaled stinger emitting the odour. It is of interest that IPA inhibits bees from scenting with the Nasonov gland. Hence, queenless workers can find their queen by Nasonov scenting, but release sting alarm pheromones upon finding a foreign queen, and this IPA is used to promote aggression against an alien queen. Does this explain why a new queen must be introduced for several days to a colony before she will be accepted?

Drones produce a pheromone that attracts other flying drones to promote the formulation of drone aggregations at sites suitable for mating with virgin queens. There are BPs. The presence of brood (both larvae and pupae) in a colony inhibits the ovarian development in worker bees. Hence, you rarely find evidence of a laying worker in a colony that still has live brood. Further, nurse bees can readily distinguish between worker and drone larvae, and pupae are correlated with the presence of brood-recognition pheromones.

The behaviour and success of a honeybee colony is often determined by the intensity and frequency to which individuals respond to these stimuli and thereafter perform tasks (Pankiw et al. 1994). Honeybees, being highly social insects, are divided into two female castes within a colony: the queen and several thousand workers. The queen is the main reproductive and head of the colony—which she controls by means of primer/releaser pheromones—and mediates aspects of worker behaviour as well as partially inhibiting worker reproduction. The workers, on the other hand, perform non-reproductive tasks such as brood rearing, nest guarding and foraging. This caste differentiation ultimately causes behavioural and physiological differences, which in turn result in differences in the composition of pheromones released. These differences can be seen in the composition of the mandibular and Du-four's glands as well as in alarm and aggregation pheromones. Honeybee pheromones can be divided into two broad categories, namely primer and releaser pheromones. Primer pheromones are those that trigger or suppress developmental events, whereas releaser pheromones are known to cause a change in the behaviour of the recipient. This chapter addresses the castespecific differences in pheromone blends and how this correlates to functionality within the colony.

7.3 Interactions Between Queens and Worker Bees

Slessor et al. (2005) reported many aspects of pheromone communication in the honeybee. The authors described in detail the components of the QRP that is attractive to worker bees. It is known that QRP entices worker bees to lick and antennate the queen

to gather a small sample of this attractive blend. The essential component of this blend is 9-oxo-(E)-2-decenoic acid (ODA). Other components include two enantiomers of ODA's biosynthetic precursor, (R)- and (S)-9-hydroxy-(E)-2-decenoic acid (HDA), and two aromatic components methyl *p*-hydroxybenzoate (HOB) and 4-hydroxy-3-methoxyphenylethanol (HVA). All these compounds are products of the mandibular glands and the blend is called the queen mandibular pheromone (QMP). Individual components are not by themselves attractive; only when all five components are combined does the blend elicit the full retinue response. It has become evident that some strains of honeybees do not find synthetic QMP at all attractive; thus, there exist additional substances involved in the retinue response. Methyl oleate, coniferyl alcohol, palmityl alcohol and linolenic acid have been identified as further synergistic substances. Three new fatty-acid-derived constituents are not of mandibular gland origin, and therefore the terminology shift to QRP was necessary. The complete identity of the QRP is still not fully defined. Except for methyl oleate (a synergistic component of QRP), other queen esters (the palmitates, oleates, ethyl stearate, ethyl and methyl palmitoleate) have been found. These queen esters are distributed as passive chemical passengers in the queen bouquet, because they are not attractive. They function as primer pheromones, which affect the physiology of the worker bees. For example, ethyl palmitate is apparently an active agent contributing to the queen's ability to inhibit worker ovarian development (Slessor et al. 2005). Primer pheromones are efficient means for maintaining social harmony in the colony and their effects are important. These pheromones act by affecting the physiology of the recipients with a subsequent shift in their behaviour (Le Conte and Hefetz 2008). Many primer pheromones also have a releaser effect. For example, QRP acts as a releaser pheromone by attracting worker bees to the queen and as a primer pheromone by physiologically inhibiting worker ovary development (Wanner et al. 2007). Primer functions are associated with brood and QRPs (Pankiw 2004a). The most abundant queen mandibular gland pheromone component, 9-keto-2-(E)-decenoic acid (9-ODA) and two aromatic components, HVA and HOB, are similarly transmitted (Naumann et al. 1991, 1992). Thus, the queen mandibular gland pheromone complex is transferred through the nest as a unit. After being secreted onto the body surface of the queen, it is removed by worker bees in the queen's retinue, especially those who come into contact with the queen through their mouthparts. Other worker bees acquire pheromones via direct contact with retinue bees or with other worker bees that have already acquired the queen pheromone. Grooming behaviour also contributes to the transfer of pheromone. Naumann (1991) showed in his study that self-grooming results in the translocation of synthetic queen mandibular gland pheromone from the mouthparts and head to the abdomen and limbs of honeybee workers. The queen mandibular gland pheromone can also reach worker bees through queen or worker "footprints" onto comb wax. Fischer and Grozinger (2008) tested the effects of QMP exposure on starvation resistance, lipid storage, and gene expression in the fat bodies of worker bees. QMP can indeed modify nutrient storage pathways, because QMP-treated bees survived much longer compared with control bees when starved and also had higher lipid levels. Expression of vitellogenin RNA, which encodes a yolk protein, was also higher in the fat bodies of QMP-treated bees. Bees involved

in brood care (nurses) have higher lipid stores than forager bees. QMP thus slows the transition from nursing to foraging. It also regulates the timing of colony-level reproduction (swarming). This mechanism is described by Pankiw (2004a). QMP also inhibits queen-rearing behaviours. When the titre of QMP decreases below inhibitory thresholds, worker bees initiate queen rearing. As colonies grow, the worker population increases in size and the amount of QMP which reaches individual bees decreases due to a dilution effect and restricted movement. Worker bees are released from the inhibitory effects of QMP on queen rearing and begin to rear queens in preparation for swarming. Queen pheromones regulate the reproductive division of labour; specifically, the queens prevent the reproduction of workers. The presence of the brood has the same effect. Dietemann et al. (2006) found that an invasive lineage of parasitic Cape honeybee (*A. m. capensis*) worker bees occurring in the range of *A. m. scutellata* has resistance to reproductive regulation by host queens. Worker reproduction in *A. m. capensis* is associated with the production of queen-like pheromones. *A. m. scutellata* queens do not prevent the production of queen-like mandibular gland compounds by parasites. Parasitic worker bees produce these signals despite the presence of a queen. This mechanism allows *A. m. capensis* worker bees to usurp resources and reproduction in foreign colonies. In the study of Kocher et al. (2009), it was suggested that the queen pheromone blend is modulated by the reproductive status of the queens. Worker bees of appropriate bee colonies can detect these subtle differences and are more responsive to queens with higher reproductive potential. Queen honeybee pheromone modulates many aspects of worker physiology and behaviour and is critical for colony social organisation. This pheromone is produced in the mandibular glands and it differs between virgin and mated, laying queens. In comparison with virgin queens, naturally mated queens, and queens experimentally inseminated with either semen or saline the following differences were found: naturally mated queens had the most activated ovaries and the most distinct chemical profile in their glands, whereas the ovary activation and chemical profiles of other experimental queens (instrumentally inseminated queens and virgins) were distinct. Pheromone samples were collected 2 days after mating or insemination. Natural mating probably has considerable importance, because the experimentally inseminated queens were intermediate between virgins and naturally mated queens, whereas no significant differences between semen- and saline-inseminated queens were found. Thus, the results suggest that the insemination process or fluid volume is responsible for stimulating these early post-mating changes in honeybee queens. In all aculeate hymenopteran females, the poison gland and the Dufour gland is associated with the sting apparatus (Abdalla and Cruz-Landim 2001). Secretion from the Dufour gland is caste regulated. Martin and Jones (2004) found that C28–C38 esters are associated with queens and alcohol eicosenol is associated with non-laying worker bees. Both esters and eicosenol are biochemically similar compounds (both are products of fatty acid biosynthesis). Egg-laying worker bees in queenless colonies produce both esters and eicosenol. Egg-laying anarchistic worker bees and parasitic Cape worker bees from queenright colonies even show the typical queen pattern (i.e. esters present and eicosenol absent). Dufour's gland pheromone operates apparently as a fertility signal. Dor et al. (2005) conducted experiments in which two worker

bees were confined in a small arena. Between worker bees, a hierarchy of reproductive dominance was established, i.e. one worker demonstrated greater ovarian development than her paired bee. Ovarian development was tightly linked to the production of queen-like Dufour's gland secretion. There was a particular increase in the production of queen-like esters. Their occurrence can serve as a reliable fertility signal. Advertising ovarian status may recruit helper worker bees with less developed ovaries to assist their nestmates. Gilley et al. (2006) used the method of solid-phase micro-extraction (SPME, 65 μ m PDMS-DVB fibre) to sample the volatile compounds emitted by live honeybee queens and workers. They detected nine compounds and four of these were present only in queens. One of these four queen-specific compounds, identified as E- β -ocimene, was expressed fully only in established mated queens and may signal the diploid egg-laying activity. The three remaining compounds (including one identified as 2-phenylethanol) were associated with unmated queens. The five compounds that the authors detected in both queens and workers were hydrocarbons and apparently function in social recognition.

7.4 Interactions Between Queens and Drones

An elaborate system of chemical communication in honeybees has evolved primarily in the context of social behaviour and mating. Single components (or a mixture of components) of the queen mandibular gland secretion may have different functions. For example, the virgin queen uses the mandibular gland secretions to attract drones on her mating flights, whereas the mated queen uses mandibular gland secretions to signal her presence to worker bees in the hive (Brockmann et al. 2006). In the 1970s, 9-ODA, the major component of the mandibular gland secretions, was shown to function as a sex pheromone. 9-ODA is viewed as the major long-distance sex attractant. Drones and virgin queens leave their colonies for mating flights. Drones gather at the drone congregation areas (estimated sizes range from 50 to 200 m in diameter) and wait there for virgin queens. Upon detection of the pheromone, drones initiate searching and chasing the queen with only a few fast ones being successful (Brockmann et al. 2006). Wanner et al. (2007) identified an odorant receptor (Or) for the queen's 9-ODA in drone antennae. They assayed the pheromone responsiveness of four candidate receptors (AmOr10, -11, -18, and -170) by using *Xenopus* oocytes and electrophysiology. AmOr11 responded specifically to 9-ODA ($EC_{50} = 280 \pm 31$ nM) and not to any of the other seven QRP components (9-HDA, HOB, HVA, methyl oleate, coniferyl alcohol, 1-hexadecanol, and linolenic acid). 9-ODA is probably the only QRP component that acts as a long-distance sex pheromone (Wanner et al. 2007). Brockmann et al. (2006) suggested that other components of the queen's secretion play a role in the communication between sexes. (2E)-9-hydroxydecanoic acid (9-HDA) and (2E)-10-hydroxydecanoic acid (10-HDA) apparently act over a short range. These two compounds are not attractive to drones from a distance, but added to 9-ODA they increased the drone's contacts with a queen dummy. A similar increase in the number of drones making contact with the baited dummy was also

found when tergite gland extracts were added to 9-ODA (Brockmann et al. 2006). The tergal gland secretion is composed of long-chain fatty acids (major compound is (Z)-9-octadecenoic acid), long-chain esters (predominant decyl decanoate was detected in virgin queens) and a linear series of unsaturated hydrocarbons (Wossler and Crewe 1999). Tergal gland alkenes probably do not function as sex pheromones. The production of queen tergal gland alkenes starts after mating. Smith et al. (1993) demonstrated in their experiments that the production of tergal gland alkenes is stimulated by natural mating and not by experimental insemination. It has long been recognised in the beekeeping industry that instrumentally inseminated queens are not as productive as naturally mated queens. Problems are observed with initial introduction and acceptance of the inseminated queens; rapid replacement of the introduced inseminated queen by a queen raised from her eggs and decreased brood production by inseminated queens. The tergal gland alkenes may play a key role in the care and acceptance of the queen and her eggs by worker bees in the hive (Smith et al. 1993). Rhodes et al. (2007) recorded changes in constituent levels from head extracts of queen with increasing age. Non-mated 7-day-old queens had higher average levels of 9-HDA, 9-ODA and 10-HDA than mated 7-day-old queens. These results suggest that these particular three constituents have sex pheromone functions in the honeybee. 9-octadecenoic acid and decyl decanoate from the tergal gland may also participate in the communication between sexes.

7.5 Interactions Between Worker Bees

One key advantage of eusociality is shared defence of the nest, brood and stored food (Breed et al. 2004). Defence of the nest plays an important role in the biology of honeybees. Defensive behaviour is partly induced and modulated by pheromones. These alarm pheromones are produced in the mandibular gland and sting apparatus of worker bees (Pankiw 2004a). Most honeybee alarm pheromone components are produced in the Koschewnikow gland and sting gland (Breed et al. 2004). More than 40 compounds (including precursor, intermediate and final biosynthetic products) have been identified from extracts of the worker sting apparatus (sting gland and Koschewnikow gland; Pankiw 2004a). About 15 components release one or more alarm behaviours (flying from the nest to locate the source of disturbance, pursuing, biting and stinging; Pankiw 2004a). IPA was first identified as a defensive compound (Boch et al. 1962). IPA elicits more stinging activity than any of the other defensive compounds and it also acts as a target-marking pheromone, guiding other defenders to the sting site (Pankiw 2004a). Pickett et al. (1982) identified a less volatile component, (Z)-11-eicosenol, as another effective alarm pheromone component for inducing stinging behaviour. (Z)-11-eicosenol prolongs the activity of the more volatile IPA presumably by slowing down the evaporation of IPA. The blend of IPA and (Z)-11-eicosenol is active for a longer time than IPA alone (Pickett et al. 1982). Hunt et al. (2003) analysed the alarm pheromone components from colonies of Africanised honeybees and they found an unsaturated derivative of IPA (3M2BA).

This compound was present at levels of 0–38 % the amount of IPA. Behavioural assays showed that 3M2BA recruited worker bees from hives at least as efficiently as IPA (Hunt et al. 2003). IPA and 3M2BA are synergistic in their natural ratios and a mixture of these two compounds recruited bees more efficiently than either of the compounds alone (Hunt et al. 2003). 3M2BA may be specific to certain populations of Africanised honeybees (Breed et al. 2004). The mandibular glands of worker bees also produce the alarm substance, 2-HPT (Pankiw 2004a). This compound shows a much lower ability to attract guards from colony entrances and sting than IPA does (Breed et al. 2004). With increasing age of worker bees, the size of the mandibular gland and the amount of 2-HPT progressively increases (Vallet et al. 1991). This means that the level of 2-HPT is higher in foragers than in guard bees. It is therefore possible that 2-HPT has other functions associated mainly with foraging behaviour. It showed a repulsive effect when added to sucrose solution which was visited by foragers and it may act as a repellent forage-marking scent (Vallet et al. 1991; Giurfa 1993). Repellent scents are used to avoid the probing of flowers that have recently been depleted of nectar or pollen (Stout and Goulson 2001). Worker bees strongly reject all flowers they have recently visited (Giurfa 1993). Flowers just abandoned by the other worker are also rejected, in a lower although significant proportion (Giurfa 1993). Differences in the response level of bees to their own marks or to partner's marks suggest that repellent scent marks are primarily self-use signals (Giurfa 1993). However, Stout and Goulson (2001) also observed interspecific interactions. Bumblebees (*Bombus lapidarius*, Apidae) avoided flowers recently visited by honeybees and vice versa. Honeybees rejected flowers that had previously been visited by bumblebees even more than those previously been visited by honeybees. The repellent forage-marking scents of bumblebees are tarsal secretions (long-chain alkanes and alkenes), which are less volatile than 2-HPT (Goulson et al. 2000; Stout and Goulson 2001). The molecular weight of 2-HPT is 114, whereas bumblebee tarsal hydrocarbons have a molecular weight of ca. 300–400 (Stout and Goulson 2001). Stout and Goulson (2001) also found that repellent forage-marking scents can be active for 40–60 min. This suggests that honeybees may use less volatile substances than 2-HPT and it is possible that 2-HPT is not the only repellent forage-marking scent that they use. If bumblebees and honeybees both use tarsal secretions as repellent scents, bumblebees, being larger than honeybees, may deposit larger quantities. This may cause a higher frequency of rejection of flowers previously visited by bumblebees. Foraging honeybees apparently also use long-term attractant scent marks. Stout and Goulson (2001) found that honeybees visited flowers that had been visited in the previous 24 h more often than flowers that had never been visited (the effect of repellent forage-marking scents disappeared and nectar was replenished in flowers). Honeybees apparently use scent marks—Nasanov secretions and (Z)-11-eicosenol—as attractants to mark rewarding flowers. The Nasanov gland of the worker honeybee is located just beneath the sixth intertergal membrane, near the dorsal surface of the abdomen (Wells et al. 1993). When a bee raises its abdomen and flexes the terminal segment, the intertergal membrane is exposed and volatile secretions of the Nasanov gland are released (Wells et al. 1993). Many of the components of the Nasanov pheromone are

biochemically related (Slessor et al. 2005). In this mixture were identified nerol ((Z)-3,7-dimethyl-2,6-octadien-1-ol), geraniol ((E)-3,7-dimethyl-2,6-octadien-1-ol), (E) and (Z)-citral ((E/Z)-3,7-dimethyl-2,6-octadienal), nerolic acid ((Z)-3,7-dimethyl-2,6-octadienoic acid), geranic acid ((E)-3,7-dimethyl-2,6-octadienoic acid) and (E, E)-farnesol ((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol; Free et al. 1981). These terpene derivatives contribute to the characteristic odour of several plant species (for example, lemon-grass; Wells et al. 1993; Slessor et al. 2005). It was found that the Nasanov scent elicits clustering activity during swarming (Abdullah et al. 1990). When the swarm leaves the nest, the bees form an unstructured cloud that remains within 50 m of the old nest. Worker bees settle in various spots and form small, incipient clusters until the queen joins one of the clusters. Worker bees rapidly crowd around the queen and emit attraction signals from their Nasanov glands, so that the cluster with the queen attracts bees from the other queenless clusters (Janson et al. 2005) and the swarm gradually forms into a tight cluster. The Nasanov pheromone is associated with a variety of circumstances. It is used to recruit nestmates to a new nesting cavity in a swarming context, to mark the entrance of the nest (helping to orient lost or dislocated worker bees) and to mark profitable food and water sources (Wells et al. 1993; Sandoz et al. 2007). Honeybees are well known for their “dance language” (Sandoz et al. 2007). Waggle dances are mechanical signals that are used by returning foragers to recruit other foragers to food, water and nest cavities (von Frisch 1967). In the communication of honeybees, mechanical and chemical signals dominate, since they must be easily perceived by bees in the darkness that prevails inside the hive (Seeley 1998). Thom et al. (2007) found that waggle-dancing bees produce and release four cuticular hydrocarbons (two alkanes, tricosane and pentacosane, and two alkenes, Z-(9)-tricosene and Z-(9)-pentacosene) from their abdomens into the air. These compounds are produced subcutaneously (they are not stored within a gland) and are present in only minute quantities on the surface of the cuticle in non-dancing worker bees (Thom et al. 2007). When these substances are injected into a hive, they significantly increase the number of foragers leaving the hive (Thom et al. 2007). This suggests that these compounds may play a pheromonal role in worker recruitment. A primary characteristic of eusocial life is the division of labour (Pankiw 2004b). In a colony of honeybees, there is a typical age-related division of labour among the worker bees, in which individuals perform different tasks at different ages (Robinson and Huang 1998). Worker bees generally perform different tasks in the nest (i.e. cell cleaning, brood rearing, comb building, nectar ripening, caring for the queen and drones, etc.) for the first 3 weeks of adult life and then venture outside to collect food and defend the nest when they get older (Kolmes et al. 1989; Huang and Robinson 1992; Robinson and Huang 1998). But, division of labour in honeybee colonies is not rigid (Robinson and Huang 1998; Leoncini et al. 2004). Worker bees can accelerate, delay and even reverse their behavioural development in response to changes in colony or environmental conditions (Huang and Robinson 1992, 1996). Removing older bees (foragers) from a colony accelerates the behavioural development of younger bees (some bees initiate foraging when they are as young as 5 days of age), whereas adding foragers delays behavioural development, and removing nurses reverses foragers to nursing tasks (Huang and Robinson

1996; Leoncini et al. 2004; Pankiw 2004b). A major influence in a worker's developmental rate is provided by her older sister foragers whose presence inhibits the behavioural maturation of younger bees (Huang and Robinson 1996; Slessor et al. 2005). Leoncini et al. (2004) have identified a substance produced by adult forager honeybees, ethyl oleate (EO), which acts as a chemical inhibitory factor, delaying age of onset of foraging. EO was detected in different body parts (head, thorax, crop and the rest of the abdomen) of nurses and foragers (Leoncini et al. 2004). Foragers had approximately 30 times more EO in their crop (foregut specialised for storage of nectar and honey) than nurses did, despite comparable levels in the head, thorax and the rest of the abdomen (Leoncini et al. 2004). This suggests that EO is transmitted via trophallaxis, the transfer of food by mouth from one individual to another (Crailsheim 1998; Leoncini et al. 2004). Forager bees bring nectar, the main source of carbohydrates. They give the content of their crop preferentially to younger hive-mates, to food-storer bees that can again pass on a portion to other bees, but mainly deposit it into cells (Crailsheim 1998). This nectar is processed into honey.

7.6 Interactions Between Adults and Brood

In the communication between brood and worker bees, a chemical cue on the surface of larvae called brood pheromone (BP) is important (Le Conte et al. 1995; Pankiw et al. 2008). BP is a blend of ten fatty-acid esters (methyl palmitate, methyl oleate, methyl stearate, methyl linoleate, methyl linolenate, ethyl palmitate, EO, ethyl stearate, ethyl linoleate and ethyl linolenate; Le Conte et al. 1990; Le Conte et al. 2001). Some components are more active than others, but all ten individual compounds show some releaser pheromone effect on adult bees (Le Conte et al. 2001). The esters are present in different amounts and proportions as a function of caste and larval age (Le Conte et al. 1994/1995). Thus, nurses can recognise the various needs of larvae and provide them with optimal care (Le Conte et al. 2006). Four esters, methyl linolenate, methyl linoleate, methyl oleate and methyl palmitate, induce the worker bees to cap the cell with a thin cover of wax (Le Conte et al. 1994). They are produced in large quantities by the larvae during the capping (Le Conte et al. 1994, 1994/1995). Le Conte et al. (1995) tested BP as an additional chemical stimulus in the artificial rearing of the queens. They found that methyl stearate increases the acceptance of the queen cells, methyl linoleate enhances the amount of royal jelly deposited by the worker, and methyl palmitate increases the weight of the larvae. In addition to releaser effects on various aspects of brood care, BP also has primer effects (Le Conte et al. 2001, 2006). Methyl palmitate and EO increase the activity of the hypopharyngeal glands, which produce proteinaceous material that is fed by nurse bees to larvae (Mohammedi et al. 1996; Le Conte et al. 2001). BP also inhibits ovary development in worker bees similarly to the queen's pheromone (Mohammedi et al. 1998; Le Conte et al. 2001). It even seems that the presence of the unsealed brood provides an inhibitory signal stronger than the queen's pheromone (Kropacova and Haslbachova 1971; Mohammedi et al. 1998). Mohammedi et al. (1998) showed

that among the ten esters, ethyl palmitate and methyl linolenate are the compounds that are involved in the prevention of ovary development of bees. All of the ten esters (boiling point around 200 °C), generally known as BP, are non-volatile and their movement is likely facilitated by worker to worker contact (Pankiw 2004a; Maisonnasse et al. 2010). Very recently, a new highly volatile molecule, E- β -ocimene, has been identified in larvae (Maisonnasse et al. 2009). This BP component also acts as a primer pheromone with two actions on worker bee physiology: inhibition of worker ovaries (Maisonnasse et al. 2009) and acceleration of worker bee behavioural maturation (Maisonnasse et al. 2010). E- β -ocimene (boiling point 73 °C), which belongs to the terpene family, is volatile and therefore has an aerial transmission and is easily dispersed within the colony (Maisonnasse et al. 2010). All worker bees in the nest can be in direct contact through this signal, such as the nurse (young bee), also the middle-aged bees, from ages 12–21 days, that specialise in nectar processing and nest maintenance, but do not engage in the brood care (Johnson 2010; Maisonnasse et al. 2010). E- β -ocimene could be the signal for the transition of middle-aged bees to foragers (Maisonnasse et al. 2010). Brood ester pheromone (the blend of 10 methyl and ethyl esters) also modulates the behavioural maturation of worker bees and its effects vary with dose (Le Conte et al. 2001; Maisonnasse et al. 2010). Low doses of brood ester pheromone accelerate foraging ontogeny, whereas high doses of this pheromone have the opposite effect (it slows down the progression of young bees towards the tasks typical of older bees; Le Conte et al. 2001; Maisonnasse et al. 2010). Young and old larvae emit different quantities of pheromones. E- β -ocimene is emitted principally by the young instars (L1, L2–3), whereas brood ester pheromone reaches a maximum value during the capping stage (L4–5; Maisonnasse et al. 2010). The young larvae (low need in nurses) promote foraging by emitting a low quantity of brood ester pheromone and a large amount of E- β -ocimene. In contrast, old larvae (high need in nurses), by producing a high quantity of brood ester pheromone, promote tending (keeping nurses in contact with them for a longer time; Maisonnasse et al. 2010).

7.7 Mandibular and Dufour's Glands of Asian Honeybees (Primer Pheromones)

7.7.1 *The Mandibular Glands of Honeybees*

Suwannapong et al. (2010) stated that the role of honeybee mandibular gland compounds is poorly understood, although they may act as alarm pheromones. They measured forager and guard bee antennal responses evoked by two major components of mandibular gland secretions of the Asiatic honeybee, *A. cerana*. Membrane potentials of antennal sensilla were measured after exposure to three concentrations of the synthetic alarm pheromones 2-HPT and (Z)-11-eicosen-1-ol

using a potentiostat (EA161) connected to an e-corder (ED401) with microelectrodes. The resting membrane potential of *A. cerana* foragers and guards was -55.23 ± 1.44 and -56.41 ± 1.21 mV, respectively. The membrane potential of foragers after exposure to 1.0, 5.0 and 10.0 % 2-HPT was -5.32 ± 0.46 , -8.41 ± 1.33 and -11.53 ± 2.16 mV, respectively. The membrane potential of guards was -5.49 ± 1.66 , -8.46 ± 1.32 and -7.31 ± 3.46 mV, respectively. Exposure of foragers to 1.0, 5.0 and 10.0 % (Z)-11-eicosen-1-ol induced membrane potentials of -24.00 ± 6.56 , -36.36 ± 5.18 and -14.60 ± 8.20 mV, respectively; for guards they were -47.62 ± 1.46 , -46.08 ± 0.87 and -9.35 ± 1.96 mV, respectively. The highest membrane potential was found in foragers exposed to 1.0 % 2-HPT. The membrane potentials of foragers were higher than that of guards except at the highest concentration (10.0 %) of both pheromones. These findings suggest that antennal sensory receptors of foragers may have higher specific thresholds than those of guards.

In most cases, both female castes are known to produce a pheromonal blend of several compounds. Mated *A. mellifera* queens produce two aliphatic compounds, 9-hydroxy-2-(E)-decanoic acid (9-HDA) and 9-keto-2-(E)-decanoic acid (9-ODA) and two aromatic ones: HOB and HVA (Winston and Slessor 1992). Workers have 10-hydroxy-2-(E)-decanoic acid (10-HDA), 10-hydroxydecanoic acid (10-HDAA) and their respective diacids, 2-(E)-decenedioic (C10:1 DA) and decanedioic (C10:0 DA). Having said this, both castes are able to produce aliphatic compounds of the other castes in small quantities. Queens have some 10-HDA and 10-HDAA (Crewe 1982), whereas workers have trace amounts of (9-HDA; Plettner et al. 1995). QMP is known to elicit both short- and long-term behaviours. Short-term behaviours include retinue formation, swarm stabilisation (Morse 1963; Winston and Slessor 1992) and drone attraction (Butler and Faurey 1964); whereas in the long term the inhibition of queen rearing is controlled through QMP (Butler 1954a, b; Pain 1954; Winston et al. 1990, 1991).

Compounds found in the mandibular glands of *A. mellifera* workers are known to be involved in food preservation and larval nutrition. This discussion on *A. mellifera* and *A. cerana* creates a primary idea of pheromones in honeybees. Plettner et al. (1997) went on to compare the mandibular compounds of *A. mellifera* with those of *A. dorsata*, *A. florea*, *A. andreniformis* and *A. cerana*. Queens of these four Asian honeybee species contain 9-ODA and 9-HDA, whereas only *A. cerana* contains detectable amounts of HOB. The worker acid, 10-HDA, was found in all four Asian honeybee species, whereas 10-HDAA was detected only in *A. cerana* and *A. florea*. Workers of all species had 10-HDA, 10-HDAA, C10:0 DA and 9-HDA in their mandibular glands, whereas *A. cerana* was the only species with no detectable C10:1 DA and 9-ODA. With respect to quantitative differences in the total production of mandibular gland secretion, *A. cerana* and *A. dorsata* produce substantially more than European *A. mellifera*—200–300 mg compared with 150–200 mg, respectively (Billen and Morgan 1998). Plettner et al. (1997) showed quite clearly that differences in mandibular gland secretions occur between open- and cavity-nesting species. Queens of open-nesting species do not contain any aromatic compounds or 10-HDAA, whereas the workers of cavity-nesting species contain 9-ODA, a typical

queen mandibular gland compound. Within the open-nesting group of honeybees, the dwarf bees, *A. florea* and *A. andreniformis*, although closely related phylogenetically (Alexander 1991), show differences within mandibular gland secretions. For example, workers show differences in their 10-HDAA/C10:0 DA profiles, whereas both castes have different profiles for 9-HDA/9-ODA. Interestingly, the queens of the Asian cavity-nesting species, *A. cerana*, do not produce HVA, which is necessary for *A. mellifera* to produce full retinue behaviour. As *A. cerana* queens elicit a full retinue response without the HVA compound, one might assume that a primer response is gained either through other chemical cues or additional non-chemical cues, as yet to be identified. In summary, it appears that all species of *Apis* analysed till date have distinct blends of compounds in the mandibular glands of both castes. Furthermore, within each species, queen and worker variation is evident. Phylogenetic studies (Alexander 1991; Dyer 1991; Raffiudin and Crozier 2007) indicate that the more ancestral, open-nesting bees have a simpler queen mandibular blend containing only aliphatic components as well as smaller differences between queen and worker secretions (Plettner et al. 1997). This may be an indication that the components and functionality of the mandibular gland have played a significant role in the evolutionary history of the genus *Apis* (Plettner et al. 1997).

7.7.2 The Dufour's Gland Pheromones

The Dufour's gland was first described by Dufour in 1841 (Trojan 1930) and is attached to the sting apparatus in all female hymenopterans, an area generally used for defensive behaviour by workers and reproduction by queens (Katzav-Gozansky et al. 2002; Martin and Jones 2004). In line with its anatomical placement, the Dufour's gland has been hypothesised to have many functions including the toxic enhancement of venom (Carlet 1890), a lubricant for the moving parts of the sting, neutralisation of the excess acid secretion in the sting (Trojan 1930; Kerr and de Lello 1962), a protective coating for the eggs (Billen 1987) and an egg-marking pheromone to aid worker policing (Ratnieks 1995).

Martin and Jones (2004) studied chemical changes in Dufour's gland secretions associated with ovary development in several species of honeybees—*A. mellifera*, *A. cerana*, *A. andreniformis* and *A. florea*. They found that C21–C33 hydrocarbons were present in all individuals, whereas C28–C38 compounds were only associated with queens, and eicosenol, a C20 unsaturated alcohol, is only associated with non-laying workers. At a finer scale, C28–C38 esters are associated only with cavity-nesting honeybee queens, whereas eicosenol, a worker alarm pheromone, is associated only with their non-laying workers. Three main chemical groups secreted by the Dufour's gland are hydrocarbons, esters and eicosenol.

Dufour's gland has been shown to have caste biosynthetic plasticity in *Apis* species (Katzav-Gozansky et al. 1997a; Sole et al. 2002) and is metabolically active throughout an individual's life (Katzav-Gozansky et al. 2000). This chemical plasticity, therefore, raises doubts about there being castespecific biosynthetic pathways.

Katzav-Gozansky et al. (2002) found that the glandular expression of the Dufour's gland chemicals is regulated by two factors, firstly, a social factor, i.e. the presence or absence of the queen and/or the interactions between nest members; and secondly, worker physiology, i.e. caste, task and reproductive state of the individual bees. The Dufour's gland exhibits both caste specificity (Katzav-Gozansky et al. 1997b) and biosynthetic plasticity (Sole et al. 2002), which raises questions as to its function and activity regulation that remain to be answered.

Worker-laid eggs covered with queen-like esters were policed as quickly as worker-laid eggs with no queen-like substance on them, which rules out Dufour's gland being used solely in egg-marking (Katzav-Gozansky et al. 2001, 2002). Based on what is known, it is assumed that, in the presence of a queen, the synthesis of esters in the worker caste is inhibited and, on removal of the queen, workers will produce queen-like esters. As only females with activated ovaries possess esters in their Dufour's gland secretion, a link may exist between ovary development and Dufour's gland secretion (Katzav-Gozansky et al. 2002). However, very little is known and much is in a state of speculation. Katzav-Gozansky et al. (2002) raised the following questions that need addressing: Is neuro-endocrine control involved? What is the nature of the queen signal? Does a single compound in the queens' secretions inhibit production in the workers? Is there a case for gland cross-reactivity (i.e. the mandibular gland secretion of the queen inhibits Dufour's ester production in workers)?

The evolution of eusociality requires the evolution of functional worker sterility. In many eusocial Hymenoptera, including the honeybees (genus *Apis*), workers respond to the presence of their queen and their ovaries remain completely inactive. When queenless, however, about one-third of honeybee workers will activate their ovaries and lay eggs (Jay 1968). Because of haplodiploidy, eggs laid by unmated workers are fully viable and result in fertile males. Thus, worker reproduction enables a queenless colony to rear a final batch of drones before the colony dies. It can therefore be an important component of worker fitness, and perhaps for this reason, complete worker sterility has not evolved in the honeybees (Ratnieks 1988) as it has in some ants (Wilson 1971; Fletcher and Ross 1985).

The proximate mechanisms that regulate worker ovary activation in the honeybees are well understood. Queens (Butler and Fairey 1963; Free 1987; Hoover et al. 2003) and brood (Arnold et al. 1994; Mohammadi et al. 1998) produce a variety of pheromones from multiple sources whose combined action inhibits ovary activation in workers. Chief among these are the pheromones secreted by the queen's mandibular glands (Butler 1959). Mandibular gland secretions of queens have a high proportion of 9-keto-(E)-2-decenoic acid (9-ODA), whereas the glands of workers produce secretions that are richer in (E)-10-hydroxy-2-decenoic acid (Plettner et al. 1995, 1996). Thus, the ratio of 9-ODA/(9-ODA + 10-hydroxy-(E)-2-decenoic acid (10-HDA) + 10-hydroxydecanoic acid (10-HDAA)) is a measure of the relative "queenliness" of the pheromonal signal (Moritz et al. 2000; Hoover et al. 2005; Schäfer et al. 2006), and the more 9-ODA circulating in a colony the less likely *A. mellifera* workers are to activate their ovaries (Hoover et al. 2003). However, controversy remains as to whether queen pheromones are best interpreted as an "honest

signal” of the presence of a queen (Seeley 1985; Keller and Nonacs 1993; Peeters et al. 1999) or a suppressive agent that chemically castrates workers against their personal reproductive interests (Butler and Fairey 1963; Fletcher and Ross 1985; Hefetz and Katzav-Gozansky 2004; Strauss et al. 2008). The honest signal hypothesis posits that in the presence of a fecund, queen workers have increased inclusive fitness if they do not activate their ovaries and prevent other workers from doing so (Ratnieks 1988). Queens too benefit from a clear reproductive monopoly. Thus, under the honest signal hypothesis, selection should act for queens to produce a signal that is unambiguous, and workers should be selected to respond to this signal by not activating their ovaries. In contrast, the “suppressive agent” hypothesis suggests that the queen’s signal chemically suppresses ovary activation in her workers against the worker’s reproductive interests in a form of parental manipulation (Alexander 1974). Under this hypothesis, we would predict an evolutionary arms race between workers and queens in which mutations in workers that allow worker reproduction are countered by mutations in queens to neutralise the mutations in workers (West-Eberhard 1981; Nanork et al. 2007a). Indeed, the chemical complexity of queen pheromones has been cited as evidence that such an arms race has occurred and as support for the suppressive agent hypothesis (Hefetz and Katzav-Gozansky 2004).

A. cerana and *A. mellifera* are two related species of cavity-nesting honeybees that are thought to have diverged 2–3 million years ago (Oldroyd and Wongsiri 2006). The species differ strongly in the rates of ovary activation of workers in queenright nests. In *A. mellifera*, less than 1 % of workers show any signs of ovary activation (Velthuis 1970; Taber 1980; Ratnieks 1993). In contrast, up to 5 % workers in queenright *A. cerana* colonies may have eggs in their ovaries (Oldroyd et al. 2001; Nanork et al. 2007b). Despite 2 million years of separation, components of the QMP of both species are similar (Plettner et al. 1997).

The evolutionary distance between *A. cerana* and *A. mellifera* is sufficiently small that transfers of pupae between *A. cerana* and *A. mellifera* colonies result in the acceptance of newly eclosed workers (Atwal and Sharma 1968; Tan et al. 2006), enabling the construction of artificial mixed-species colonies. Such heterospecific colonies provide a useful system in which to investigate the proximate mechanisms by which worker reproduction is regulated in insect colonies. If honeybee worker ovary activation is regulated by an honest signal of queen fecundity, then we would predict that workers in a heterospecific nest with a functional queen would respond by not activating their ovaries. This interpretation requires that the signal has been preserved over evolutionary time—which it should be because both sender and receiver benefit from the signal. Alternatively, if worker ovary activation is regulated by a suppressive agent produced by queens, then we would predict that workers in a heterospecific nest would activate their ovaries at higher frequency than in colonies of their own species, because of changes in the suppressive agents wrought by more than 2 million years of evolution. Here, we investigate these alternatives by comparing rates of ovary activation of *A. cerana* and *A. mellifera* workers in both queenless and queenright colonies of their own species and in colonies comprised of heterospecific workers. Within the heterospecific colonies, we compared rates of ovary activation when the

queen was conspecific and heterospecific, thus allowing us to untangle the effects of signals from the queen and workers.

Tan et al. (2002) reported that when a honeybee colony loses its queen, workers activate their ovaries and begin to lay eggs. This is accompanied by a shift in their pheromonal bouquet, which becomes more queen like. Workers of the Asian hive bee *A. cerana* show unusually high levels of ovary activation and this can be interpreted as evidence for a recent evolutionary arms race between queens and workers over worker reproduction in this species. To further explore this, they compared the rate of pheromonal bouquet change between two honeybee sister species of *A. cerana* and *A. mellifera* under queenright and queenless conditions. They showed that in both species, the pheromonal components HOB, 9-ODA, HVA, 9-HDA, 10-HDAA and 10-HDA have significantly higher amounts in laying workers than in non-laying workers. In the queenright colonies of *A. mellifera* and *A. cerana*, the ratios $(9\text{-ODA})/(9\text{-ODA} + 9\text{-HDA} + 10\text{-HDAA} + 10\text{-HDA})$ are not significantly different between the two species, but in queenless *A. cerana* colonies the ratio is significantly higher than in *A. mellifera*, suggesting that in *A. cerana*, the workers' pheromonal bouquet is dominated by the queen compound, 9-ODA. The amount of 9-ODA in laying *A. cerana* workers increased by more than 585 % compared with the non-laying workers, that is 6.75 times higher than in *A. mellifera*, where laying workers only had 86 % more 9-ODA compared with non-laying workers.

Tan et al. (2009) found that in dequeened honeybee colonies ovarian activation occurs in some workers, and the pheromonal bouquets of these laying workers become more queen like. In the Asiatic honeybee, *A. cerana*, we compared the amount of 9-keto-2(E)-decenoic acid (9-ODA), a mandibular gland pheromone component, between non-laying workers from queenright colonies and laying workers from queenless colonies, and further, applied synthetic 9-ODA to workers to determine whether they discriminate workers with activated ovaries based on the level of this compound. Levels of 9-ODA were higher in laying workers from dequeened colonies than in non-laying workers from queenright colonies. In both queenright and queenless colonies, workers attacked more workers treated with 9-ODA than control-treated workers. These results suggest that detection of pseudoqueens in *A. cerana* is mediated by changes in 9-ODA.

7.8 Post-Embryonic Development

The postembryonic development of optic lobes in *A. cerana cerana* was comparatively studied by using immunohistochemical method (5-bromo-2-deoxyuridine, BrdU immunostaining) and in situ cell death detection (Li Zhao-Ying and XI Ceng-Si 2008). The results showed that the medulla and lamina were generated by distinct populations of neuroblasts in the outer optic anlage (OOA), and the neuroblasts divided asymmetrically to generate ganglion mother cells. Ganglion mother cells later divided symmetrically to generate immature neurons. Generation of the medulla cortex started with the onset of the final larval instar. Generation of the lamina cortex

was initiated with the arrival of retinal afferents at the optic lobes. The lobula neurons were generated by neuroblasts in a second structure, the inner optic anlage (IOA). The results suggest that the optic lobe was generated from two optic anlagen: (1) the proliferation of neurons showed a peak at prepupa and (2) generation of the lamina cortex was initiated with the arrival of retinal afferents at the optic lobes.

7.9 Brood Pheromones

To study the effects of BP esters (methyl palmitate, ethyl palmitate and EO) on feeding and capping behaviour of workers and development of the queens of *A. c. cerana* and *A. m. ligustica*, 1-day-old worker larvae were transferred to the artificial queen cells made of bee wax that were mixed with three aliphatic esters at concentrations of 1 % and 0.1 % (w/w), and then the acceptance of queen cells, weight of each larva, and weight of royal jelly in each queen cell were tested (Zeng Yun-Feng et al. 2010). Dummy larvae made of paraffin mixed with three aliphatic esters at the concentrations of 1 % and 0.1 % (w/w), respectively, were introduced into the empty cells, and the worker acceptance of cells was tested. After dripping royal jelly mixed with three aliphatic esters at the concentrations of 1 % and 0.1 % (w/w), respectively, into the queen cells when the larvae was 1-, 2- and 3-day-old, the emergence weight and the quantity of ovarioles of queen were tested. The results showed that (1) methyl palmitate (0.1 %) significantly improved the weight of larvae in each queen cell in both *A. c. cerana* and *A. m. ligustica*; (2) methyl palmitate (1 %, 0.1 %) and EO (1 %, 0.1 %) significantly increased the capping rate of the dummy larvae in *A. m. ligustica* and (3) EO (1 %, 0.1 %) significantly depressed weight and ovarioles of the queen in both *A. c. cerana* and *A. m. ligustica*. These results indicate that different BPs of honeybee have different biological effects.

Yan Wei-yul et al. (2009) to analyse the brood kairmone and pheromone of different stage brood in *A. c. cerana* and compare it with *A. mellifera*. Worker larvae (2-, 6- and 7-day old) and drone larvae (2-, 7- and 8-day old) of *A. c. cerana* were extracted with hexane by crushing, the supernatant were then fractioned by chromatography on a column of silica gel and tested by gas chromatogram. It is the first time to identify the components of brood kairmone and pheromone from *A. c. cerana* are methyl palmitate, methyl oleate, methyl stearate, methyl linoleate, methyl linolenate, ethyl palmitate, EO, ethyl stearate, ethyl linoleate and ethyl linolenate. The kairmone and ten aliphatic esters in brood have been found in both *A. mellifera* and *A. c. cerana*, whereas the content and distribution of the two pheromone are different at different brood stages.

Table 7.2 Alarm pheromone components. (Source: Christian et al. 2011)

Substance	Species	Reference
Iso-pentyl acetate	<i>A. m.</i> , <i>A. c.</i> , <i>A. d.</i> , <i>A. f.</i>	Morse et al. (1967), Koeniger et al. (1979)
1-Hexanol	<i>A. m.</i>	Collins and Blum (1983)
Octyl acetate	<i>A. m.</i> , <i>A. c.</i>	Schmidt et al. (1997), Wager and Breed (2000)
Butyl acetate	<i>A. m.</i>	Hepburn et al. (1994)
1-Butanol	<i>A. m.</i>	Collins and Blum (1983)
1-Octanol	<i>A. m.</i>	Collins and Blum (1983)
2-Methyl-1-butanol	<i>A. m.</i>	Wager and Breed (2000)
3-Methyl-1-butanol	<i>A. m.</i>	Wager and Breed (2000)
Hexyl acetate	<i>A. m.</i>	Boch et al. (1962)
2-Decen-1-yl acetate	<i>A. d.</i> , <i>A. f.</i>	Veith et al. (1978), Koeniger et al. (1979)
2-Nonanol	<i>A. m.</i> , <i>A. c.</i>	Collins and Blum (1983), Schmidt et al. (1997)
(Z)-11-eicosen-1-ol	<i>A. m.</i> , <i>A. c.</i>	Pickett et al. (1982), Schmidt et al. (1997)
Eicosenol	<i>A. m.</i> , <i>A. c.</i>	Schmidt et al. (1997)
Benzyl acetate	<i>A. m.</i>	Hepburn et al. (1994)

A. m. *Apis mellifera*, *A. c.* *Apis cerana*, *A. d.* *Apis dorsata*, *A. f.* *Apis florea*

7.10 Alarm, Aggregation and Other Pheromones of Asian Honeybees (Releaser Pheromones)

All known species of honeybees show a well-developed and coordinated defensive behaviour (Butler 1954c) involving several behavioural mechanisms to fend off potential threats (e.g., shimmering and hissing—Fuchs et al. 2001, “testudo” behaviour—Pirk et al. 2002, both in *A. florea*) and if this were not sufficient, it escalates to multiple stinging events. This defensiveness was originally described by Huber (1814) who stated that the attacks were enhanced, and probably triggered, by the odour of the venom. However, it has been subsequently shown that neither the venom, the venom glands nor the sac plays a role in producing this odour (cf. Schmidt et al. 1997). IPA was originally shown to be an important component of the alarm pheromone (Boch et al. 1962; Free and Simpson 1968); however, as chemical methods of analysis improved over the years, more substances (approximately 30) were identified (Table 7.2). The amounts of IPA produced vary between species, but do not seem to be correlated with honeybee size. *A. dorsata* produce the most (21.8 mg), followed by *A. mellifera* (1.9 mg) and thereafter by *A. cerana* and *A. florea* with only 0.2 mg (Koeniger et al. 1979). Koeniger et al. (1979) showed that all four species react to the extract of the sting apparatus from each of the species, but to a lesser degree and that the reaction could not be correlated with the amount of IPA produced/used. Schmidt et al. (1997) dissected the sting apparatus of *A. mellifera*, *A. florea*, *A. dorsata* and *A. cerana* and analysed their venoms using GC-MS and showed that they contain large, oily droplets, lacking in the other three species. Furthermore, *A. cerana* seems to possess 50–100 times more eicosenol than *A. mellifera*, plus long-chain alcohols and hydrocarbons, which were found in oily droplets of the venom. Schmidt et al. (1997) suggested that the oily droplet plays a major role as an alarm pheromone in *A. cerana*. It might also play a role as a carrier for other active

components which in turn trigger defensive behaviour (Veith et al. 1978; Pickett et al. 1982). The suggestion that it might serve as an alarm component on its own (Pickett et al. 1982) was not supported by results ranking it as inactive in eight of nine assay categories including releasing stinging (Free et al. 1989). It only inhibited the release of the Nasonov pheromones by scenting guards. Additional differences between the two sister species is not only the amount of eicosenol, but also the anatomical source as well. In *A. mellifera*, most of it is found in the setose area, whereas in *A. cerana* large quantities are found in the venom itself (Schmidt et al. 1997). Three hypotheses have been put forward to explain its function: it plays a role in marking food sources or marking enemies, or as a carrier facilitating the spreading of aqueous venom toxins. Martin and Jones (2004) proposed that the biosynthesis of esters and eicosenol in Dufour's gland is caste regulated and, following the argument by Pickett et al. (1982), that it is a worker alarm pheromone.

Another component found in the sting extract, which does not occur in all species, is 2-decen-1-yl acetate (2-DA). It was only found in the extracts of *A. florea* and *A. dorsata* and has the specific effect of extending the duration of the alarm reaction when presented in combination with IPA compared with pure IPA (Koeniger et al. 1979). The unusual venom of *A. cerana* contains large oily droplets within an otherwise aqueous secretion. Chemical analysis (GC-MS) revealed that the venom oil consists of (Z)-11-eicosen-1-ol (81.2 %), other linear alcohols (7.7 %) and linear hydrocarbons (11.1 %). The eicosenol is present in extremely large quantities, averaging more than 250 mg per insect, and is absent, or present in small quantities, in other parts of the sting apparatus. An investigation of the site of eicosenol storage in *A. mellifera* showed it to be absent from the venom and to be associated with the setose area where the more volatile components of the alarm pheromone are stored, as previously shown by others. A third group with the eastern bees from the mainland, from India to Japan, was less well supported.

Koeniger et al. (1979) studied the behavioural responses to sting extracts among the four species and observed minor differences in the duration of the reaction to the extract. In *A. mellifera* and *A. cerana*, it lasted ca. 3 min and in *A. florea* and *A. dorsata* 6–15 min. Interspecific tests led to the conclusion that *A. florea* and *A. dorsata* must have an additional pheromone besides IPA. Using gas chromatographic analysis, 2-DA as found in sting extracts of *A. florea* and *A. dorsata*. 2-DA had a specific effect in *A. florea* and *A. dorsata* only. It extended the duration of the alarm reaction compared with the reaction on pure IPA. A mixture of IPA and 2-DA had a similar effect on the behaviour and reaction time, as did sting extracts.

The unusual venom of *A. cerana* contains large oily droplets within an otherwise aqueous secretion (Schmidt et al. 1997). Chemical analysis (GC-MS) revealed that the venom oil consists of (Z)-11-eicosen-1-ol (81.2 %), other linear alcohols (7.7 %), and linear hydrocarbons (11.1 %). The eicosenol is present in extremely large quantities, averaging more than 250 g per insect, and is absent, or present in small quantities, in other parts of the sting apparatus. An investigation of the site of eicosenol storage in *A. mellifera* showed it to be absent from the venom and to be associated with the setose area where the more volatile components of the alarm pheromone are stored, as previously shown by others. A third honeybee species, *A.*

dorsata, does not contain the alcohol. The function of eicosenol in *A. cerana* is not clear, but may serve to mark stung intruders with pheromone or to attract foragers to marked floral resources.

7.11 Aggregation Pheromones

The components released by the Nasonov gland are partially responsible for individual honeybees staying together in a swarm and forming a cohesive unit. A complex of seven different components (Pickett et al. 1980) is released from the dorsal surface of the abdomen mostly accompanied by fanning behaviour to attract fellow workers (Free 1987) either in swarm preparation or in organising the defence of the colony. Three of the components are particularly responsible for the lemon-like smell—geraniol, geranial and neral. In *A. florea*, exposure of the Nasonov gland appears not to be accompanied by fanning (Free and Williams 1979), added to which no evidence could be found that *A. florea* uses this gland to scent mark foraging sources as Butler (1954c) reported when he observed that workers exposed their Nasonov gland when visiting flowers.

Abdullah et al. (1990) extracted Nasonov gland secretions of Asiatic honeybee *A. cerana* using three different methods and found that (E)-citral and geraniol were determined quantitatively, and (Z)-citral was also present in trace amounts. The amount of (E)-citral found was 20.0 µg/bee when pheromone extraction was by the abdomen dipping (in hexane) method, but only 3.8 µg/bee using hexane extraction of the excised glands. Geraniol (2.5 µg/bee) was detected only by the latter method. The clustering response of worker bees to the following individual (synthetic) compounds and to mixtures of them was tested: 9-oxo-2-decenoic acid, geraniol, nerol, farnesol, (E) and (Z)-citral. The last one was the most effective in causing clustering.

7.12 Impact of Genetic and Environmental Effects

The development of animals depends on both genetic and environmental effects to a varying extent (Tan 2007). Their relative influences can be evaluated in the social insects by raising the intracolony diversity to an extreme in nests consisting of workers from more than one species. In this study, we studied the effects of mixed honeybee colonies of *A. mellifera* and *A. cerana* on the rearing of grafted queen larvae of *A. cerana*. *A. mellifera* sealed worker brood was introduced into *A. cerana* colonies and on emergence, the adults were accepted. Then, *A. cerana* larvae were grafted for queen rearing into two of these mixed-species colonies. Similarly, *A. cerana* larvae and *A. mellifera* larvae were also grafted conspecifically as controls. The success rate of *A. cerana* queen rearing in the test colonies was 64.5 %, surpassing all previous attempts at interspecific queen rearing. After emergence, all virgin queens obtained from the three groups ($N = 90$) were measured morphometrically.

The *A. cerana* queens from the mixed-species colonies differed significantly in size and pigmentation from the *A. cerana* control queens and closely approximated the *A. mellifera* queens. It is inferred that these changes in the *A. cerana* queens reared in the mixed-species colonies can be attributed to feeding by heterospecific nurse bees and/or chemical differences in royal jelly. Our data show a strong impact of environment on the development of queens. The results further suggest that in honeybees the cues for brood recognition can be learned by heterospecific workers after eclosion, thereby providing a novel analogy to slave-making in ants.

7.13 Differences Between Bees

Christopher et al. (2001) analysed the head extracts of workers and mated queens of the closely related species of *A. cerana* and *A. nigrocincta* from Sulawesi, Indonesia were quantitatively analysed by gas chromatography-mass spectrometry for several mandibular gland components. The amounts of many compounds were significantly different between species for both queens and workers. Quantities of 10 of the 16 compounds quantified in queen bees differed significantly between the two species. Of the three known mandibular gland retinue pheromone components in *A. cerana* queens (E)-9-oxodec-2-enoic acid (9-ODA), (E)-9-hydroxydec-2-enoic acid (9-HDA) and HOB, the amounts of 9-HDA and HOB were significantly different between species. Quantities of 6 of the 11 compounds quantified in worker bees differed significantly between the two species. This quantitative analysis supports the hypothesis that *A. cerana* and *A. nigrocincta* are indeed separate species

7.14 Honeybee Communication in Relation to Foraging

Foragers communicate their floral findings in order to recruit other worker bees of the hive to forage in the same area. The factors that determine recruiting success are not completely known, but probably include evaluations of the quality of nectar and/or pollen brought in to the hive. Honeybees communicate to each other by two ways: the physical communication by the dance language and the chemical communication by means of pheromone and/or odour that transmit important information to members of the honeybee colony. Pheromones play an important role in recruitment communication (Free 1987). They use pheromones to guide nestmates for food sources, warn them of danger signal and mark territory area (Leal 2010). Honeybee can smell or detect odour or chemical signal such as pheromone, flower odour, nectar by sensory receptor located on the flagellum of their antennae (Suwannapong et al. 2010a, b).

7.14.1 Foraging Behaviour Using Forage: Marking Pheromone

Honeybees have sophisticated foraging coordination and communication (von Frisch 1971; Suwannapong 2000). This activity is only performed by workers, known as foragers or foraging bees. Some foragers specialise on pollen foraging and some on nectar foraging. Between these extremes, there are a large number of generalists who collect both (Fewell and Page 1993). The range for the onset of foraging ranges from 18.3 days (Sakagami 1953) to 37.9 days of age (Winston and Fergusson 1985). This food consists of carbohydrates and proteins (nectar and pollen; Seeley 1985). Under normal conditions, worker bees begin to forage when they are about 2–3 weeks old. Foraging is the last chore in the life of a worker. Part of the colony's stored honey is consumed by foraging bees who need fuel and therefore consume a certain amount of honey to ensure that she will have a sufficient energy supply for her round-trip journey (Akranakul 1976; Seeley 1985). To obtain a full load of nectar and pollen (or both) in a single trip, she may have to visit several hundred flowers (Akranakul 1976). The amount of energy she expends, related to the amount of food she collects, is determined largely by factors such as the amount of nectar obtained per flower, floral density per unit area, the distance from the hive and weather conditions (Akranakul 1976; Partap 1992; Partap and Partap 1997).

It has been also reported that *A. mellifera* foragers use 2-HPT to mark previously visited flowers, thereby signalling nectar depletion to other bees (Engels et al. 1997; Giurfa 1991). However, the four native Thai *Apis* species do not appear to use aversive pheromone marking during foraging (Suwannapong 2000; Suwannapong et al. 2010c). For example, they may revisit the same flower briefly after the first visit and continue to forage on the same flower simultaneously with several bees of their own species or other species. Suwannapong (2000) observed *A. florea*, two to three bees of *A. cerana*, one to two bees of *A. dorsata* and one to two bees of *A. andreniformis* visiting the same flower (Suwannapong 2000). It is also possible that honeybees, similar to bumblebees, can learn to associate floral depletion or floral reward using olfactory cues such as cuticular hydrocarbon “footprints”, which are deposited while walking on the food source (Leadbeater and Chittka 2007).

However, this remains to be investigated. The mandibular gland of *A. mellifera*, the source of this putative food-marking pheromone is primarily 2-HPT. However, the primary component of mandibular gland secretions in Thai honeybees is (Z)-11-eicosanol. In general, the ten most abundant components in the mandibular glands of all these species are 80 % similar (Suwannapong 2000).

7.14.2 Forage Marking Pheromones

Most honeybee pheromones are produced by exocrine glands, which are ectodermal glands of the epidermis that secrete to the outside of the body. Each pheromone consists of odorants with a mixture of low molecular weight that move through the air

and are perceived by bee antennae. Some pheromones and semiochemicals are perceived through direct contact with the antenna (Haynes and Millar 1998). Honeybee mandibular glands are pheromone producing exocrine glands whose secretions may function as alarm pheromone, which is an important component of colony defence (Blum 1969). The mandibular glands are largest relative to body size in queens, large and well-developed in workers, and very small in drones. The secretory product of workers mandibular glands has an oily appearance, and its major component is 2-HPT, a volatile substance that accumulates in the central reservoir (Engels et al. 1997).

The function of worker mandibular gland pheromone is unclear. At high concentrations, this pheromone may be repellent (Balderrama et al. 1996). Shearer and Boch (1965) reported that 2-HPT is the main compound of worker mandibular gland and acts as a secondary alarm pheromone in *A. mellifera* guards. Maschwitz (1964) suggested that mandibular glands produce alerting pheromones, although a less effective one than the sting apparatus pheromones. Shearer and Boch (1965) identified 2-HPT from mandibular gland secretions. Guard bees were alerted by, and attacked, filter paper carrying 2-HPT placed at the hive entrance. Boch and Shearer (1971) therefore suggested that 2-HPT has two functions: alarm (with lower efficacy than the sting gland) and repelling workers when deposited on exhausted floral resources. However, Nieh (2010) reported that foragers collecting food exhibited no alarm behaviour in response to mandibular gland extracts, although they were clearly alarmed by sting gland extract. The response of other *Apis* species to worker mandibular gland pheromone is similarly unclear. Guards and foragers of *A. florea* and *A. cerana* showed diverse responses to (Z)-11-eicosen-1-ol, the main component of mandibular gland pheromone in these species (Suwannapong et al. 2010). Moreover, the flower-marking hypothesis is not consistent with the finding that 2-HPT can attract foragers at low concentrations (Shearer and Boch 1965; Boch and Shearer 1971; Kerr et al. 1974; Vallet et al. 1991), as would occur on flowers shortly after pheromone deposition.

Worker *Apis* mandibular gland pheromone may play a key role in two important aspects of colony life, defence and foraging, but its function in honeybees remains unclear. Understanding the function of this pheromone is significant because of the importance of studying honeybee forage marking pheromone in terms of practical apiculture; for understanding how honeybees use pheromones to mark nectar depleted flowers to save energy; for describing the role of pheromone as an attractant when a new and rich food source is discovered; and for application to increase honeybee pollination. For example, if the pheromone (such as QMP) is an attractant, it can be applied in the field to attract bees to pollinate certain plant crops, which there are now commercial examples that are used to increase crop yields (Currie et al. 1992a; Currie et al. 1992b). On the other hand, if worker mandibular gland pheromone is a repellent, it may be useful in repelling bees where they are not wanted.

7.15 Role in Management

With a better understanding of pheromones, a beekeeper would have much less swarms, have higher forager populations, increased honey production, receive less stings, learn to manage bees without wearing all those hot protective clothes, and find the joys of beekeeping! If all those things are not enough to excite you to learn more about pheromones, I suggest that you find another less scintillating hobby such as stamp collecting or identification of rocks or something else that is dead rather than vibrantly alive, because of your knowledge and aid to honeybee survival.

7.16 Future Needs

There is a tremendous amount of research yet to be done regarding the role of pheromones in the life of the honeybee colony, but the hard part, i.e. just knowing that pheromones play a very important role in the lives of honeybees, is already done. Now, it is just a matter of finding the money to support pheromonal research among honeybees in our schools of scientific learning today.

7.17 Conclusion

The success of honeybees is mainly based on their ability to communicate efficiently, which enables them to explore highly temporal food sources and organise a rapid response to disturbances, and which are crucial for the self-organisation of a colony. Chemical communication, particularly in honeybees, is based on the fact that pheromones are released under context-dependent circumstances. For example, who releases the pheromone and who is on the receiving end and which class of pheromones is used will depend on the circumstances (Le Conte and Hefetz 2008). These multi-functional properties of pheromones make them ideal tools to investigate underlying evolutionary mechanisms of speciation. In the *Apis* complex, *A. mellifera*, *A. cerana*, *A. florea*, *A. dorsata* and *A. nuluensis* give us an insight as to how a neutral mutation might have resulted in different functional adaptations, as all species show similarities, but with added significant differences, for example the case of the (Z)-11-eicosen-1-ol in *A. cerana*. Moreover, interspecific comparisons of the abundance of different chemical compounds, more than 30 of which are involved in an alarm response, could provide us with more information and understanding of the evolution of pheromonal communication and its proximate mechanisms. As Table 7.2 indicates, most of the honeybee pheromonal work has been done with *A. mellifera*. Comprehensive and comparative approaches in investigating the similarities and differences within and between the balance of the Asian honeybee species is lacking and needs further investigation.

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Chapter 8

Molecular Phylogeny of *Apis cerana*

8.1 Introduction

There has been a long fascination of human beings for the complexity of social insect colonies and the industrious nature of their workers. The major organizing principle of ant, bee and termite societies is reproductive division of labour whereby one or a few individuals, the queens, specialize in reproduction while the others, the workers, participate in co-operative tasks such as building the nest, collecting food, rearing the young and defending the colony. This social organization provides numerous advantages and is the basis for the tremendous ecological success of social insects (Hölldobler 1990). The sequencing of the honeybee genome (The Honeybee Genome Sequencing Consortium 2006) is an exciting step towards uncovering the molecular events underlying the evolution of altruism and complex behaviours.

A genome sequence, like a honeybee queen, is useless if it is not accompanied by the assiduous labour of a large number of co-operative workers. For this reason, an industrious swarm of scientists has collaborated to conduct detailed analyses and comparisons of the honeybee genome with those of model organisms, in particular the fruit fly *Drosophila melanogaster*. These analyses, reported in no fewer than 40 companion papers published in *Science*, *PNAS* and special issues of *Genome Research* and *Insect Molecular Biology*, have revealed many interesting features associated with the unusual social biology of the honeybee (Wurm et al. 2007).

To date, most of our understanding of behavioural genetics has come from studies in model organisms such as *D. melanogaster* and *Caenorhabditis elegans*. In only a handful of cases, however, have genetic variants been shown to be responsible for behavioural differences observed under natural conditions (Osborne et al. 1997; Keller and Ross 1998). An interesting feature of the honeybee is that workers change tasks as they age. They typically remain in the nest when young and then switch to dangerous tasks outside the nest only when they are older. This switch can be manipulated by altering colony needs or by treatment with chemicals that cause precocious foraging. Capitalizing on this, Whitfield et al. (2006) conducted a series of clever experiments to separate the effects of worker age, genotype, environment and experience on gene expression. They found that the transition from hive work to

foraging is accompanied by a robust molecular signature with many genes sharing the same expression pattern across the conducted experiments. Examination of these genes revealed shared *cis*-regulatory promoter elements that may be responsible for their co-regulation (Sinha et al. 2006). This is a big step towards clarifying the regulatory cascades governing the networks of behavioural genes. Future investigations into the neuronal circuitry of bees and its modulation should also be facilitated by the bioinformatic and proteomic identification of 36 prohormones coding for more than 200 neuropeptides (Hummon et al. 2006).

Comparison of the honeybee genome to that of other insects revealed a number of interesting differences. Not a complete surprise was the identification of nine new genes linked to the production of royal jelly, which workers feed to the queen and larvae. These genes apparently evolved from a single progenitor gene which encodes a member of the ancient Yellow protein family (Drapeau et al. 2006). Similarly, the striking expansion of the odorant receptor family in honeybees (170 genes) relative to *D. melanogaster* (62 genes) and the mosquito *Anopheles gambiae* (79 genes) (Robertson and Wanner 2006) makes sense given the prime role of pheromones in communication and the need for workers to discriminate between diverse floral odours. These discoveries should help elucidate some of the bees' fascinating skills, which include precise memory of space and odours as well as the abstract modelling and linguistic abilities shown by the 'waggle dance'.

A more surprising finding was that honeybees have only half as many immune defence genes as *D. melanogaster* or *A. gambiae*. Many authors have suggested that colonies of social insects should be under particularly strong pathogenic pressure because numerous highly related individuals live in close quarters. Although not frequently recognized, however, bees spend most of their lives in a protected colony environment, while flies and mosquitoes grow up in rotting food or stagnant water. Moreover, the food provided to bee larvae has already been processed by adults and thus is less likely to contain pathogens. Likewise, the risk of poisoning or infection may be low when foraging nectar and pollen from flowers with which a mutualism has evolved (Evans et al. 2006). The close association between bees and plants, and the lack of incentive for plants to produce toxic nectar or pollen may actually also explain the a priori surprising finding that honeybees have far fewer gustatory receptors than *D. melanogaster* and *A. gambiae* (Robertson and Wanner 2006). Another possible explanation for why bees have fewer immune defence genes is that they display social behaviours such as extensive grooming and 'social fever' that may effectively combat infections (Evans et al. 2006).

The honeybee genome has provided several interesting revelations concerning the most unusual characteristic of social insects: their ability to produce very different phenotypes from the same genotype as a result of the alternative developmental programs followed by queens and workers. Indeed, in several ant species, queens can differ dramatically in size and morphology from workers, yet almost nothing is known about the epigenetic factors underlying the developmental switch responsible for these differences. Of particular interest was the discovery of 65 microRNAs, including some that show caste-specific expression patterns during development (The Honeybee Genome Sequencing Consortium 2006). This raises the exciting

possibility that microRNAs are involved in caste determination via differential gene expression between queens and workers.

The finding that the honeybee genome harbours genes encoding a complete set of methyltransferases, the highest known eukaryotic CpG content, and the evidence for CpG methylation of protein-coding genes (PCGs) are of great interest, given that methylation of CpGs represses transcription in mammals (Table 8.4). Interestingly, in contrast to mammals, in the honeybees, DNA methylation was detected predominantly in coding regions. Perhaps methylation plays a role in regulating genes involved in developmental differences between honeybee queens and workers (Wang et al. 2006). Finally, it appears that more than 60 genes are duplicated specifically in the honeybee, including 2 genes for components of the insulin pathway (The Honeybee Genome Sequencing Consortium 2006). This pathway regulates growth in other animals (Wu and Brown 2006) and could be the means through which queen bees become bigger than workers (Wheeler et al. 2006). These and/or other duplicated genes may be involved in caste or sex determination and differences, and/or in social interactions.

Comparative analyses revealed some other peculiar genomic features including the fact that the honeybee genome evolves at a much slower rate than the strongly derived genomes of flies and mosquitoes. This is evidenced by sequence identity, intron conservation, and gene loss relative to an ancestor common to insects and vertebrates (The Honeybee Genome Sequencing Consortium 2006). The slow evolution of the honeybee genome may be general to hymenopteran insects, whose haplo-diploid sex-determination system might purge deleterious mutations that would be masked in diploid individuals (The Honeybee Genome Sequencing Consortium 2006). Alternatively, it could be due to the long generation time of social insects—queens typically can live many years (Keller and Genoud 1997)—and/or to the low effective population size resulting from a single individual monopolizing reproduction in the hive.

Interestingly, these characteristics may also be responsible for another idiosyncrasy of the honeybee, the very high average recombination rate of 5.7 recombinations per chromosome (Beye et al. 2006). Such a high recombination rate has only been reported in one other species, the ant *Acromyrmex echinator* (Sirvio et al. 2006). High recombination rates might thus be a characteristic of social insects, again perhaps a result of their typically long generation time and small population size. Alternatively, high recombination rates might have been selected as a means to increase genetic diversity among offspring.

Another striking finding was that the honeybee genes controlling circadian rhythm and telomere length are more similar to vertebrates than to *D. melanogaster* or *A. gambiae* (The Honeybee Genome Sequencing Consortium 2006). *Drosophila* is considered the paradigm of insect biology. This clearly needs to be changed given the increasing evidence that many of *Drosophila*'s features, such as their early-acting axis specification genes, are highly derived and not characteristic of insects.

The honeybee genome sequence and attendant analyses and experiments open many avenues for future research. In anticipation of a finished genome, the BeeSpace project has begun the dissection of environmental and hereditary influences on brain

gene expression in the context of defensive behaviour and foraging using thousands of microarrays. Beyond division of labour and co-operation within a colony, it will be exciting to understand the molecular basis of the evolution of within-colony conflicts and their resolution. Functional tests, through ectopic expression or repression of genes involved in both behaviour and caste development, will be essential in elucidating how insect societies function.

At a more basic level, the mechanisms by which solitary species became social could be pinpointed by examining either facultatively social species or contrasting pairs of solitary and primitively social species. The independent evolution of social life in bees, ants, wasps and termites also provides a unique opportunity to determine whether the convergent morphological, physiological and behavioural adaptations that have occurred in these taxa are due to modification of the same developmental pathways and gene networks. The recent development of an expressed sequence tag (EST) library, microarray and other molecular genetic tools for the fire ant, *Solenopsis invicta*, should pave the way for such sociogenomic comparative studies.

Biodiversity of the honeybee was first assessed using morphometrics based on an extensive study and multivariate analyses (Ruttner et al. 1978). However the analysis of mitochondrial DNA (mtDNA) has become widely used approach in studying the biogeography of the honeybees. One region of the *Apis* mitochondrial genome (mt genome), an intergenic region (coding and non-coding sequences) located between cytochrome oxidase I (COI) and cytochrome oxidase II (COII) has proved to be particularly informative for intraspecific studies because the sequences do not appear to be subject to strong purifying selection and accumulate numerous base substitutions and insertion/deletions (Cornuet et al. 1991). It has been used widely in the study of the phylogeny and biogeography of *Apis* species (Smith and Hagen 1996; De La Rúa et al. 2000; Smith et al. 2000). Variation in this sequence shows a strong geographic pattern and indicates several mtDNA lineages within *Apis cerana* (Hepburn et al. 2001; Smith et al. 2004). Earlier phylogeographic studies using this mtDNA sequence indicates that there are at least four major lineages of *A. cerana* (Smith et al. 2004). These are Sundaland (including samples from Peninsular Malaysia, Bali, Lombok, south Thailand, Singapore, Java, Flores, Borneo, Timor and Palawan), Mainland Asian (including samples from north and central Thailand, Korea, Japan, China, India, Burma, Nepal, and Pakistan), Oceanic Philippine lineages from the Philippine Islands and Yellow Indian bees from India and Sri Lanka (Smith et al. 2005). The Indian honeybee *A. cerana* occurs in south India in two distinct colour morphs of bees: the Yellow (Plain) and Black (Hill) morphs which are collectively known as *A. cerana*. The Hill morph is associated with a widely distributed mitochondrial haplotype that is present throughout the Mainland populations of Southeast Asian *A. cerana*. In contrast, the Plain morph, which is apparently confined to low to moderate elevations in India and Sri Lanka, is associated with a unique mitochondrial haplotype that is not present in other cavity-nesting honeybees. By comparison, *A. cerana* has been reported to have a great deal of geographic variations among its populations (Smith and Hagen 1996); studies of the mtDNA of *A. cerana* and other Asian honeybees are very few (De La Rúa et al. 2000; Smith et al. 2005; Cornuet and Garnery 1991; Baskaran and Thiyagesan 2010; Ferreira et al. 2009).

The eastern cavity-nesting honeybee, *A. cerana* Fabricius, is widespread over Asia and occupies a distribution extending from Afghanistan to China and from Japan to southern Indonesia (Ruttner 1988). Based on morphometric analyses, this species has been grouped in four subspecies with different distribution ranges (Ruttner 1988): *A. cerana cerana* from Afghanistan, Pakistan, north India, China and north Vietnam; *A. cerana indica* from south India, Sri Lanka, Bangladesh, Burma, Malaysia, Indonesia and the Philippines; *A. cerana japonica* from Japan and *A. cerana himalaya* from central and east Himalayan Mountains (Smith 1991b). These subspecies include many populations, some of which are geographically isolated, such as those in the Philippines archipelago. As on other oceanic islands, these populations may have undergone evolutionary changes giving rise to reproductively isolated populations.

Over the past 20 years, mtDNA studies have shed light on the biogeography of the Asian cavity-nesting honeybee *A. cerana*. One region of the *Apis* mt genome, a non-coding sequence located between cytochrome oxidase I (COI) and cytochrome oxidase II (COII) has proved to be particularly informative for intraspecific studies because the sequences do not appear to be subject to strong purifying selection and accumulate numerous base substitutions and insertion/deletions (Cornuet et al. 1991). Variation in this sequence shows a strong geographic pattern, and indicates several mtDNA lineages within *A. cerana* (De La Rúa et al. 2000; Deowanish et al. 1996; Hepburn et al. 2001; Sihanuntavong et al. 1999; Sittipraneed et al. 2001a, b; Smith 1991a; Smith and Hagen 1996, 1999; Smith et al. 2000). Mitochondrial lineages include Mainland Asian (including samples from India, Nepal, northern Thailand, Korea, Japan, and Hong Kong and Kunming, China), Sundaland (including samples from peninsular Malaysia, Java, Bali, Lombok, Flores, Timor and Borneo), and a Philippine group (including samples from Luzon and Mindanao). In addition, a distinctive mitochondrial haplotype is found in the so-called yellow or plains bees from India and Sri Lanka. Deowanish et al. (1996) studied the mtDNA variation of *A. cerana* from Japan, Korea, Taiwan, Vietnam, Thailand, Nepal and the Philippines, and examined it by restriction fragment length polymorphism (RFLP) analysis. Using ten restriction enzymes, they could discriminate among different localities including groups from: (1) Japan; (2) Nepal, Vietnam and north to central Thailand; (3) Korea-Tsushima; (4) Taiwan; (5) south Thailand and (6) Philippines. *A. cerana*, a honeybee native to Asia, was classified into four subspecies by Ruttner (1988) on the basis of morphometric and geographic distribution. *A. cerana himalaya* ranges from the Southeast Asian mountains through Nepal to Thailand and probably Southwest China. *A. cerana cerana* ranges through Afghanistan, Pakistan, north India, northern and eastern China and north Vietnam. *A. cerana indica* occurs from south India, Sri Lanka, Bangladesh, Myanmar, Malaysia, southern Thailand, Indonesia and the Philippines, while *A. cerana japonica* is restricted to Japan. mtDNA analysis has proved to be most powerful tool, relative to *A. mellifera* (Garner et al. 1991, 1992), for identifying population diversity. Variation in mtDNA of *A. cerana* was first studied by Smith (1991a, 1993) who proposed three groups: a Mainland Asian group including Japan, Thailand, Malaysia, Borneo and south India; a group from Luzon; and a group from the Andaman Islands. This result differed from the morphological identification (Ruttner 1988) which separates *A. cerana japonica* from the other

groups. Since the mtDNA study examined few samples from some locations and no samples from the vast region of China and the Himalayas, it cannot be expected to fully explain phylogenetic relationship of *A. cerana* populations.

Smith et al. (2004) investigated genetic variation and biogeography of the cavity-nesting honeybee, *A. cerana*, in Burma to add them to the larger picture of *A. cerana* biogeography in Asia. Non-coding regions of mtDNA of 23 colonies collected from 12 localities were sequenced to identify their genetic lineages. Six haplotypes were found (Japan1, Nepal1, ThaiS1, BurmaN1, BurmaN2 and BurmaN3) belonging to two *A. cerana* mtDNA lineages: Mainland Asian and Sundaland. The Mainland lineage was found in most parts of Burma except the southeast, where a Sundaland population was found. Studies in Thailand suggested that the Sundaland lineage was not found north of 10° 34' N; this study shows that there is a Sundaland population in Burma at 19–20° N latitude. They proposed three hypotheses to explain the presence of the Sundaland lineage in Burma: (1) Burma Sundaland bees are a relict of a formerly more widespread Sundaland population, (2) Sundaland bees migrated to this part of Burma from the southern Thai-Malay peninsula or (3) transportation by humans.

For a long time, *A. cerana* was considered a subspecies of *A. mellifera*. However, on the basis of several genetic, morphological and behavioural characteristics, it has been established that *A. cerana* and *A. mellifera* are two completely isolated species, and they are considered most recently derived and sister taxa (Cameron 1993; Engel and Schultz 1997; Yudan and Crozier 2007; Arias and Sheppard 2005). Honeybee has been the object of considerable scientific curiosity and has served as the classical model organism in diverse fields such as navigation, face recognition and sensory biology for almost a century (Somanathan et al. 2009; Dacke and Srinivasan 2007; Dyer et al. 2005). Honeybee is regarded as the premier pollinator of major fruit crops. It has a long history of association with mankind because of its cavity-nesting lifestyle and it is used for producing honey, bee pollen, wax and royal jelly (Behura 2007).

Most metazoan species possess a compact, circular mt genome, which varies in size from 14 to 19 kb that contain 36–37 genes, including 12–13 PCGs, 2 ribosomal RNAs (rRNA) genes and 22 transfer RNAs (tRNA) genes necessary for translation of the proteins encoded by the mtDNA (Boore 1999; Salvato et al. 2008; Cook 2005). mtDNA has been extensively used for studying phylogenetic and evolutionary relationships among animal species due to its maternal inheritance, rapid evolutionary rate and lack of genetic recombination (Yu et al. 2008; Hua et al. 2009; Jia et al. 2010; Li et al. 2008; Cui et al. 2007). mtDNA has proved to be an important tool in intraspecific and interspecific phylogenetic studies of honeybees (Garnery et al. 1992, 1995; Arias and Sheppard 1996; Songram et al. 2006; Cornuet et al. 1991), and one region of the *Apis* mt genome, a non-coding sequence located between cytochrome oxidase I (*cox1*) and cytochrome oxidase II (*cox2*) has proved to be particularly informative for intraspecific studies (De La Rúa et al. 2000; Chapman et al. 2008). The Hymenoptera are one of the largest insect orders belonging to hexapods, comprising over 100,000 described species (LaSalle and Gauld 1993). In 1993, Crozier reported the complete sequence of the *A. mellifera* mt genome, this was the first

Table 8.1 Mitochondrial genome sequences of Hymenoptera sequenced completely or nearly completely prior to the study and used for phylogenetic analysis. (Tan et al. 2011)

Family	Taxon	Accession number	Reference
Apidae	<i>Apis mellifera</i>	L06178	Crozier and Crozier (1993)
	<i>Bombus ignitus</i>	DQ870926	Cha et al. (2007)
	<i>Bombus hypocrita</i>	EU401918	Unpublished
	<i>Melipona bicolor</i>	AF466146	Silvestre et al. (2008)
Chrysididae	<i>Primeuchroeus</i> spp.	AH015389	Castro et al. (2006)
Pergidae	<i>Perga condei</i>	AY787816	Castro and Dowton (2005)
Vanhorniidae	<i>Vanhornia eucnemidarum</i>	DQ302100	Castro et al. (2006)
Vespidae	<i>Abispa ephippium</i>	EU302588	Cameron et al. (2008)
	<i>Polistes humilis synoecus</i>	EU024653	Cameron et al. (2008)
Pteromalidae	<i>Nasonia vitripennis</i>	AsymC EU746609	Oliveira et al. (2008)
	<i>Nasonia vitripennis</i>	HiCD12 EU746610	Oliveira et al. (2008)
	<i>Nasonia giraulti</i>	RV2 EU746611	Oliveira et al. (2008)
	<i>Nasonia longicornis</i>	IV7 EU746612	Oliveira et al. (2008)
Cephidae	<i>Cephus cinctus</i>	FJ478173	Dowton et al. (2009b)
Orussidae	<i>Orussus occidentalis</i>	FJ478174	Dowton et al. (2009b)
Ichneumonidae	<i>Venturia canescens</i>	FJ478176	Dowton et al. (2009b)
	<i>Enicospilus</i> sp.	FJ478177	Dowton et al. (2009b)
	<i>Diadegma semiclausum</i>	EU871947	Wei et al. (2009)
Evaniidae	<i>Evania appendigaster</i>	FJ593187	Wei et al. (2009)
Stephanidae	<i>Schlettererius cinctipes</i>	FJ478175	Dowton et al. (2009b)
	<i>Cotesia vestalis</i>	FJ154897	Wei et al. (2010b)
Braconidae	<i>Spathius agrili</i>	FJ387020	Wei et al. (2010b)
	<i>Phanerotoma flava</i>	GU097654	Wei et al. (2010b)
	<i>Diachasmimorpha longicaudata</i>	GU097655	Wei et al. (2010b)
	<i>Macrocentrus camphoraphilus</i>	GU097656	Wei et al. (2010b)
	<i>Meteorus pulchricornis</i>	GU097657	Wei et al. (2010b)
	<i>Aphidius gifuensis</i>	GU097658	Wei et al. (2010b)

identified mt genome of hymenopteran (Crozier and Crozier 1993). Until recently, only 9 complete and 18 nearly complete mt genome sequences have been determined in Hymenoptera (Crozier and Crozier 1993; Castro and Dowton 2005; Castro et al. 2006; Cha et al. 2007; Cameron et al. 2008; Oliveira et al. 2008; Silvestre et al. 2008; Wei et al. 2009; Dowton et al. 2009b; Wei et al. 2010a, b) (Table 8.1), and in the genus *Apis*, only the *A. mellifera* mt genome has been determined. The lack of knowledge of mt genomics for Hymenoptera is a major limitation for population genetic and phylogenetic studies of the Hymenoptera including the species in the Apidae.

mtDNA has proved to be an important tool in phylogenetic studies of insects (De-Salle 1992; Simon et al. 1994) and especially of honeybees (Cornuet and Garnery 1991; Smith 1991a; Garnery et al. 1992; Moritz et al. 1994), because the complete

sequence of the mtDNA of *A. mellifera* is available (Crozier and Crozier 1993). The mtDNA of *Apis* species has an intergenic region, between the *tRNA*-Leu gene and the second subunit of the cytochrome oxidase gene (COII) that shows length polymorphism (Cornuet et al. 1991). The nucleotide sequence of this region shows a combination of units that defines three major evolutionary lineages of *A. mellifera* mtDNA. A simple test using polymerase chain reaction (PCR) amplification of this intergenic region and subsequent restriction with the enzyme *Dra*I has been developed (Garnery et al. 1993) and used to discriminate among the mitochondrial haplotypes of *A. mellifera* subspecies and races (Moritz et al. 1994, 1998; Garnery et al. 1995; De La Rúa et al. 1998). *A. cerana* is closely related to *A. mellifera* (Garnery et al. 1991; Willis et al. 1992), and shares the presence of this intergenic region. In *A. cerana* the region is of 89 bp and has a high A-T content, which makes it suitable for the aforementioned test (Cornuet et al. 1991).

Smith and Hagen (1997) used the non-coding intergenic region of *A. cerana* to study the intraspecific biogeography of different populations sampled over the distribution range of this species. They found two types of mtDNA with western and eastern distributions, respectively, and a third, short, type with most of the intergenic region absent, which was present in two geographically distant islands (Taiwan and Sulawesi). In their phylogenetic analysis of this region they found two well-supported groups: the Sundaland group (populations from Malaysia, Borneo, Java, Bali, Lombok, Timor and Flores) and the Philippine group (populations from Luzon, Mindanao and Sangihe).

Using 21 microsatellite markers and PCR method, the polymorphisms of 20 *A. cerana* honeybee populations across China was investigated and the genetic structure and diversity of the populations were explored (Ji et al. 2011). The results showed that there were 507 alleles (mean 24.14 per locus, ranging from 13 to 45) in 842 honeybees. Wuding bee had the highest level of heterozygosity (0.695), and the lowest estimate was 0.207 for Changbai bee. The global heterozygote deficit across all populations (*Fit*) amounted to 0.776. About 42.3 % of the total genetic variability originated from differences between breeds, with all loci contributing significantly to the differentiation. An unrooted consensus tree using the neighbour-joining method and pair-wise distances showed that six populations from eastern China clustered together. The structure analysis indicated that the six populations were separated first. These findings demonstrated that the six honeybee populations had close genetic relationships with the characteristics of locus specificity, rich polymorphism, abundant and random distribution over the genome and their co-dominant inheritance; microsatellites are currently most commonly used to assess population structure and diversity (Chapman et al. 2008; Delaney et al. 2009; Soland-Reckeweg et al. 2009; Bourgeois and Rinderer 2009; Kence et al. 2009). According to Food and Agriculture Organization (FAO) recommendations, determining classic genetic distances using neutral, highly polymorphic microsatellite markers is the method of choice for investigating genetic relationships and breed differentiation. This methodology also provides information for establishing preservation priorities for livestock breeds (Barker 1999).

De La Rúa et al. (2000) in order to analyse the geographical variability of *A. cerana indica* from the Philippine Islands, studied 47 colonies from different locations in three of the larger islands (Mindanao, Luzon and Palawan) and four of the

Visayan Islands (Panay, Negros, Cebu and Leyte). Genetic variation was estimated by restriction and sequence analysis of PCR-amplified fragments of the *tRNA-Leu-COII* region. They found four different haplotypes, Ce1, Ce2, Ce3 and Ce4, that discriminate among the bee populations from different islands. The Ce1 haplotype was present in Mindanao and Visayan Islands, Ce2 restricted to Luzon, and both Ce3 and Ce4 present in Palawan only. Phylogenetic analysis of the sequences showed a great intraspecific variability in accordance with the geological history of these islands and partially agrees with some previous morphological and molecular studies.

Songram et al. (2006) studied the genetic diversity of the honeybee (*A. cerana*) in Thailand collected from north, northeast, the central region, peninsular Thailand and Samui Island by PCR-RFLP of *ATPase6-ATPase8*. Interestingly, 78 individuals (43.09 %) of the southern-latitude bees exhibited length heteroplasmy of the PCR product. The gel-eluted *ATPase6-ATPase8* (825 bp) of each bee was restricted with *TaqI*, *SspI* and *VspI*, respectively. Eight mitotypes were generated and revealed biogeographic differentiation between conspecific samples of *A. cerana*. AAA, ACA, AAD, BAA, ADA and ABA were found only in the north-to-central samples (north, northeast and central region); BBB and BBC were found in the southern-latitude bees; and BBC was restrictively found in the Samui sample. Large genetic distances were observed between each of the north-to-central samples and peninsular Thailand and Samui samples, but lower levels of genetic distance were found within each region. Geographic heterogeneity and phylogenetic analyses indicated that Thai *A. cerana* could be genetically differentiated into northern Thailand, peninsular Thailand and Samui Island populations.

Baskaran (2011a) studied the mtDNA variations in *A. cerana* populations of south India where two distinct colour morphs of bees: the Yellow (Plain) and Black (Hill) are reported to occur. In order to analyse the genetic variabilities in these morphs, the variations were estimated by the sequence analysis of PCR-amplified fragments ranging from 479 to 746 bp of *COI-tRNA-Leu-COII* region of mtDNA in *A. cerana* from selected locations of Tamil Nadu and Karnataka. Intergenic regions of mtDNA of ten colonies from different localities were sequenced to identify their genetic lineages. In this study, six haplotypes (H') were found among the ten samples sequenced. The haplotype diversity (Hd) was 0.89 (± 0.075), the variance of haplotype diversity was 0.00569 and the number of variable sites (S) were 238 among the sequenced samples in this study. Nucleotide diversity (π) was 0.281 and overall mean distance between the populations was 0.039. These morphs were associated with a widely distributed mitochondrial haplotypes that is present throughout the Mainland populations of Southeast Asia.

Baskaran (2011b) analysed mtDNA sequences to study the genetic variations and the molecular phylogenetics of the Indian honeybee *A. cerana indica* Fabricius in Tamilnadu. The PCR products were amplified and sequenced from 594 to 744 bp between cytochrome oxidase I (COI) and cytochrome oxidase II (COII) in mtDNA. It was also estimated that the average contents of Thymine (Uracil), Adenine (A), Cytosine (C) and Guanine (G) were 44.4, 40.1, 9.7 and 5.8 %, respectively among the sequenced samples. There were 247 Identical pairs (Ii), 83 Transitional pairs (Si), 304 Transversional pairs (Sv) and R (Si/Sv) was 0.27 among the pair-wise analyses

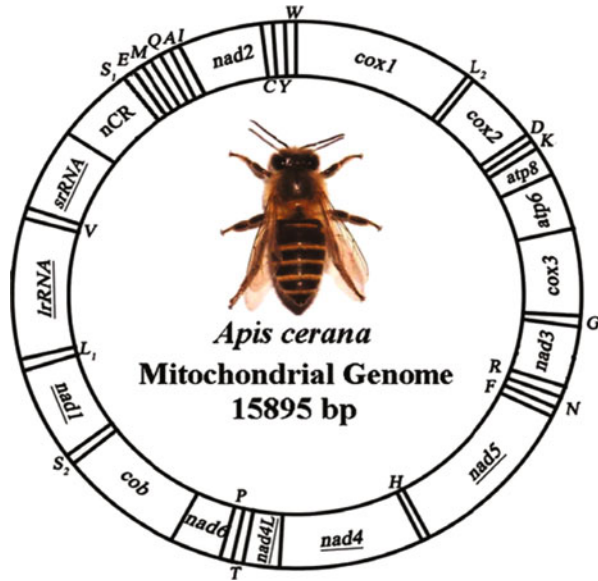
of nucleotide frequencies (%) of mtDNA of honeybee (*A. cerana indica*) of this study. Haplotype diversity 0.905 (S.D. 0.103) and nucleotide diversity 0.165 (S.D. 0.028) were calculated. Phylogenetic relationships of different geographic samples of honeybee *A. cerana indica* in Tamilnadu were obtained from the nucleotides of intergenic region between COI and COII of mtDNA.

Tan et al. (2011) determined the complete nucleotide sequence of the *A. cerana* mt genome, and performed phylogenetic analyses using selected Hymenoptera species. The new sequence may provide useful information on both genomics and the evolution of Hymenoptera, because there are only a few complete (or nearly complete) mtDNA sequences available from these animals. Tan et al. (2011) studied complete mtDNA sequence of cavity-nesting Asiatic honeybee *A. cerana*. They presented an analysis of features of its gene content and genome organization in comparison with *A. mellifera* to assess the variation within the genus *Apis* and among main groups of Hymenoptera. According to them, the size of the entire mt genome of *A. cerana* is 15,895 bp, containing 2 rRNA genes, 13 PCGs, 22 tRNA genes and 1 control region. These genes are transcribed from both strands and have a nucleotide composition high in A and T. The contents of A + T of the complete genomes are 83.96 % for *A. cerana*. The AT bias had a significant effect on both the codon usage pattern and amino acid composition of proteins. There are a total of 3,672 codons in all 13 PCGs, excluding termination codons. The most frequently used amino acid is Leu (15.52 %), followed by Ile (12.85 %), Phe (10.10 %), Ser (9.15 %) and Met (8.96 %). Intergenic regions in the mt genome of *A. cerana* are 705 bp in total. The order and orientation of the gene arrangement pattern is identical to that of *A. mellifera*, except for the position of the *tRNA-Ser*^(AGN) gene. Phylogenetic analyses using concatenated amino acid sequences of 13 PCGs, with three different computational algorithms (neighbour joining (NJ), maximum parsimony (MP) and maximum likelihood (ML)), all revealed two distinct groups with high statistical support, indicating that *A. cerana* and *A. mellifera* are two separate species, consistent with results of previous morphological and molecular studies. The complete mtDNA sequence of *A. cerana* provides additional genetic markers for studying population genetics, systematics and phylogeographics of honeybees.

8.2 General Features of the Mitochondrial Genome of *A. cerana*

The length of the complete mt genome of *A. cerana* is 15,895 bp (Fig. 8.1), and the mtDNA sequence has been deposited in the GenBank under the accession number GQ162109. The mt genome of *A. cerana* contains 13 PCGs (*cox1*–3, *nad1*–6, *nad4L*, *atp6*, *atp8* and *cob*), a small subunit rRNA gene (*rrnS*), a large subunit rRNA gene (*rrnL*), and 22 tRNA genes (Table 8.2). The *A. cerana* mt genome shows a novel arrangement within Hymenoptera compared with the ancestral pancrustacean mt genome organization (Dowton et al. 2009b). All rearranged genes are tRNAs, tRNA gene rearrangements can be classified as translocations, local inversions or shuffling. A translocation is a movement of a gene to another position across a PCG. A

Fig. 8.1 The mitochondrial genome of *Apis cerana*. Protein-coding genes (PCGs) are transcribed in a clockwise direction, except for those underlined. The two ribosomal RNA (rRNA) genes were encoded by the L strand. Transfer RNA (tRNA) genes encoded by H and L strands are shown outside and inside the circular gene map, respectively. tRNA genes are designated by single-letter amino acid codes, except those encoding leucine and serine, which are labelled L1, L2, S1 and S2, representing *tRNA*-Leu^(CUN), *tRNA*-Leu^(UUR), *tRNA*-Ser^(AGN) and *tRNA*-Ser^(UCN), respectively. (Tan et al. 2011)



rearrangement is classified as an inversion when the tRNA is found on the opposite strand and as shuffling when the tRNA gene is on the same mt stand but in a different position compared with the ancestral organization (without movement across a PCG) (Dowton et al. 2003).

As shown in Fig. 8.2, Tan et al. (2011) identified eight rearrangements shared between *A. cerana* and *A. mellifera*. *tRNA*-Ala, *tRNA*-Ser^(AGN) and *tRNA*-Glu move to *tRNA*-Ile—*tRNA*-Gln—*tRNA*-Met cluster, and *tRNA*-Ala changes position relative to *tRNA*-Ser^(AGN) and *tRNA*-Glu. *tRNA*-Trp moves across two tRNAs (*tRNA*-Cys and *tRNA*-Tyr) boundaries. The order of *tRNA*-Ile—*tRNA*-Gln—*tRNA*-Met becomes *tRNA*-Met—*tRNA*-Gln—*tRNA*-Ile, and two of tRNAs (*tRNA*-Gln and *tRNA*-Arg) arrangements are inversions. Finally, the *tRNA*-Lys and *tRNA*-Asp swap positions. The orientation and gene order of the *A. cerana* mt genome were identical to that of *A. mellifera* except for the *tRNA*-Ser^(AGN). In *A. cerana*, *tRNA*-Ser^(AGN) gene was only translocated from *nad3*-*nad5* junction to the junction of *nad2* and AT-rich region compared with the ancestral pancrustacean mt genome organization. In *A. mellifera*, *tRNA*-Ser^(AGN) gene was not only translocated but also shuffled, which swapped positions with *tRNA*-Glu gene. The duplication/random loss model, and the intramitochondrial genome recombination and duplication/non-random loss model are possible mechanisms to explain translocation and shuffling. Of these, intramitochondrial genome recombination is presumed to be more common (Dowton et al. 2003).

The genes *atp6*, *atp8*, *cox1*, *cox2*, *cox3*, *cob*, *nad2*, *nad3* and *nad6* are transcribed from one strand, while *nad1*, *nad4*, *nad4L*, and *nad5* are transcribed from the other strand. The nucleotide compositions of the entire mtDNA sequences for *A. cerana* are biased towards A and T, with T being the most favoured nucleotide

Table 8.2 Positions and nucleotide sequence lengths of mitochondrial genomes (mt genomes) of *Apis cerana*, and start and stop codons for protein-coding genes (PCGs) as well as their tRNA gene anticodons (starting from *tRNA-S_I*). (Tan et al. 2011)

Gene/region	Position		Size (bp)	Strand	Anticodon	Codon		Intergenic nucleotides ^a
	From	To				Start	Stop	
<i>tRNA-Ser</i> ^(AGN) (S _I)	1	60	60	H	TCT			3
<i>tRNA-Glu</i> (E)	64	129	66	H	TTC			34
<i>tRNA-Met</i> (M)	164	229	66	H	CAT			231
<i>tRNA-Gln</i> (Q)	461	522	62	H	TTG			0
<i>tRNA-Ala</i> (A)	523	588	66	H	TGC			18
<i>tRNA-Ile</i> (I)	607	672	66	H	GAT			0
<i>nad2</i>	673	1668	996	H		ATT	TAA	−1
<i>tRNA-Cys</i> (C)	1668	1733	66	L	GCA			5
<i>tRNA-Tyr</i> (Y)	1739	1807	69	L	GTA			16
<i>tRNA-Trp</i> (W)	1824	1892	69	H	TCA			0
<i>cox1</i>	1893	3458	1,566	H		ATT	TAA	−5
<i>tRNA-Leu</i> ^(UUR) (L2)	3454	3523	70	H	TAA			89
<i>cox2</i>	3613	4293	681	H		ATT	TAA	−2
<i>tRNA-Asp</i> (D)	4292	4359	68	H	GTC			6
<i>tRNA-Lys</i> (K)	4366	4437	72	H	TTT			6
<i>atp8</i>	4444	4605	162	H		ATC	TAA	−19
<i>atp6</i>	4587	5264	678	H		ATG	TAA	17
<i>cox3</i>	5282	6061	780	H		ATG	TAA	66
<i>tRNA-Gly</i> (G)	6128	6194	67	H	TCC			0
<i>nad3</i>	6195	6548	354	H		ATT	TAA	19
<i>tRNA-Arg</i> (R)	6568	6633	66	L	TCG			19
<i>tRNA-Asn</i> (N)	6653	6720	68	H	GTT			18
<i>tRNA-Phe</i> (F)	6739	6809	71	L	GAA			6
<i>nad5</i>	6816	8486	1,671	L		ATT	TAA	0
<i>tRNA-His</i> (H)	8487	8552	66	L	GTG			17
<i>nad4</i>	8570	9898	1,329	L		ATA	TAA	0
<i>nad4L</i>	9899	10162	264	L		ATT	TAA	23
<i>tRNA-Thr</i> (T)	10186	10252	67	H	TGT			15
<i>tRNA-Pro</i> (P)	10268	10345	78	L	TGG			50
<i>nad6</i>	10396	10905	510	H		ATT	TAA	12
<i>cob</i>	10918	12066	1,149	H		ATG	TAA	23
<i>tRNA-Ser</i> ^(UCN) (S2)	12090	12156	67	H	TGA			12
<i>nad1</i>	12169	13083	915	L		ATT	TAA	0
<i>tRNA-Leu</i> ^(CUN) (L1)	13084	13152	69	L	TAG			0
<i>rrnL</i>	13153	14480	1,328	L				0
<i>tRNA-Val</i> (V)	14481	14547	67	L	TAC			0
<i>rrnS</i>	14548	15333	786	L				0
AT-rich region (nCR)	15334	15895	562					0

^aGap nucleotides (positive value) or overlapped nucleotides (negative value) between two adjacent genes

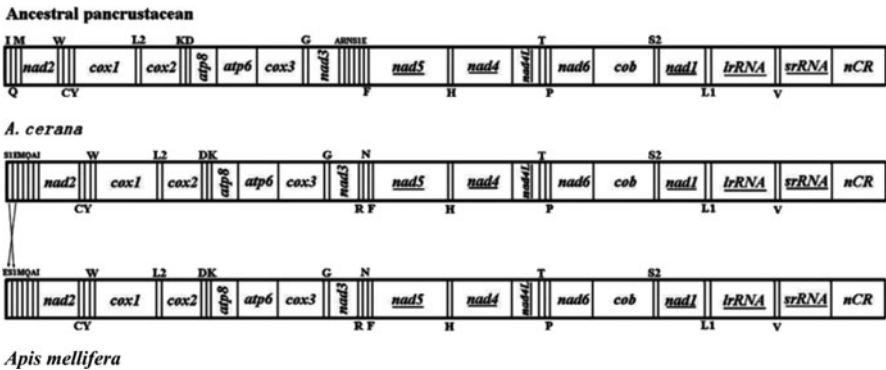


Fig. 8.2 Comparison of the mitochondrial gene arrangement among *A. mellifera*, *A. cerana* and ancestral pancrustacean. tRNA genes are labelled by one-letter symbol on the bar when transcription starts from right to left direction, or under the bar when transcription starts from left to right direction. Underlined protein-coding genes (PCGs) or rRNA genes are transcribed from right to left and PCGs that are not underlined are transcribed from left to right. (Tan et al. 2011)

Table 8.3 Nucleotide composition and skews of *Apis cerana* mitochondrial protein-coding and ribosomal RNA genes and comparison with *Apis mellifera*. (Tan et al. 2011)

Gene	Nucleotide frequency				A + T (%)	AT-skew	GC-skew	AT-skew	GC-skew
	A	G	T	C				<i>A. mellifera</i>	
<i>atp6</i>	0.366	0.056	0.472	0.106	83.8	−0.127	−0.309	−0.099	−0.269
<i>atp8</i>	0.475	0.043	0.395	0.086	87.0	0.092	−0.333	0.042	−0.413
<i>cox1</i>	0.347	0.110	0.413	0.130	76.0	−0.086	−0.085	−0.087	−0.093
<i>cox2</i>	0.385	0.087	0.404	0.125	78.9	−0.024	−0.181	−0.029	−0.167
<i>cox3</i>	0.360	0.089	0.444	0.108	80.4	−0.104	−0.098	−0.113	−0.188
<i>cob</i>	0.368	0.084	0.442	0.105	81.0	−0.091	−0.110	−0.087	−0.148
<i>nad1</i>	0.355	0.109	0.480	0.056	83.5	−0.149	0.325	−0.184	0.359
<i>nad2</i>	0.395	0.051	0.469	0.085	86.4	−0.086	−0.250	−0.091	−0.304
<i>nad3</i>	0.373	0.051	0.475	0.102	84.8	−0.120	−0.334	−0.105	−0.375
<i>nad4</i>	0.360	0.093	0.491	0.056	85.1	−0.153	0.252	−0.161	0.337
<i>nad4L</i>	0.345	0.095	0.527	0.034	87.2	−0.209	0.470	−0.234	0.676
<i>nad5</i>	0.379	0.095	0.470	0.056	84.9	−0.107	0.257	−0.115	0.297
<i>nad6</i>	0.431	0.051	0.433	0.084	86.4	−0.002	−0.246	−0.014	−0.545
<i>rrnS</i>	0.439	0.125	0.377	0.060	81.6	0.076	0.352	0.028	0.370
<i>rrnL</i>	0.404	0.111	0.428	0.058	83.1	−0.029	0.312	−0.043	0.375
Total	0.423	0.063	0.416	0.098	83.9	0.008	−0.217	0.018	−0.272

AT skew = (A % − T %)/(A % + T %); GC skew = (G % − C %)/(G % + C %)

and G the least favoured, in accordance with the mt genome of *A. mellifera*. The content of A + T is 83.9 % for *A. cerana* (42.3 % A, 41.6 % T, 6.3 % G and 9.8 % C) (Table 8.3), 84.9 % for *A. mellifera* (43.2 % A, 41.7 % T, 5.5 % G and 9.6 % C), respectively. The bias of the base composition of an individual strand can be described by skewness (Perna and Kocher 1995), which is calculated as (A % − T %)/(A % + T %) and (G % − C %)/(C % + G %), respectively. AT-skews and GC-skews of each of the

13 PCGs and the 2 rRNA genes and the whole mt genome were calculated for *A. cerana* and *A. mellifera* (Table 8.3). In *A. cerana*, except for the *atp8* gene which has a relatively weak skew of A vs. T (AT skew = 0.092), other 12 PCGs have a skew of T vs. A (AT skew between -0.002 and -0.209). The PCGs of H-strand have a strong skew of C vs. G (GC skew between -0.098 and -0.309), and the *nad1*, *nad4*, *nad4L* and *nad5* genes, which coded on the L-strand have a strong skew of G vs. C (GC skew between 0.252 and 0.470). In the two rRNA genes, the GC skew displayed the same pattern (GC skew = 0.352 and 0.312 for the *rrnS* and *rrnL* genes, respectively). The AT skew displayed an opposite pattern (AT skew = 0.076 and -0.029 for *rrnS* and *rrnL* genes, respectively). By comparing the skew values of 13 PCGs and 2 rRNA genes between *A. cerana* and *A. mellifera*, they found that GC skews of different genes coded on the same strand are all positive or negative, whereas the AT skews of different genes coded on the same strand are either positive or negative. This is congruent with the pattern of skew values in other insects (Wei et al. 2010b). Wei et al. (2010b) found that the criterion for detecting reversal of strand asymmetry on mt genomes should be the sign of GC skew values, not the AT skew values. The *A. cerana* mt genes overlap a total of 27 bp in four locations ranging from 1 to 19 bp (Table 8.2). Gene overlaps on the same strand in the mt genome of *A. cerana* can be found in three regions.

The longest is a 19-bp overlap between *atp8* and *atp6*. The second case is a 5-bp overlap between *cox1* and *tRNA-Leu^(UUR)*. The third is a 2-bp overlap that occurred between *cox2* and *tRNA-Asp*. Overlaps of genes coded by the different strands also can be found in the mt genome of *A. cerana*. Between *nad2* and *tRNA-Cys*, there exists an overlap of 1 bp. Interestingly, the *A. cerana* mt genome has the same overlapping nucleotides and locations between genes as those of *A. mellifera*, where genes also overlap a total of 27 bp in four locations (Crozier and Crozier 1993). Apart from the A + T-rich region, the *A. cerana* mt genes are separated by a total of 705 bp of intergenic spacer sequences, which are spread over 22 regions and range in size from 1 to 231 bp (Table 8.2). The longest intergenic region (231 bp) is located between *tRNA-Met* and *tRNA-Gln* genes. In *A. mellifera* mt genome, a total of 813 bp of intergenic spacer sequences are spread over 24 regions, ranging in size from 1 to 193 bp. The longest one is located between *tRNA-Leu^(UUR)* and *cox2* (Crozier and Crozier 1993). This region has proved to be particularly informative for intraspecific studies, because the sequences do not appear to be subject to strong purifying selection and accumulate numerous base substitutions and insertion/deletions (Cornuet et al. 1991). In comparison, *A. cerana* has only 89-bp intergenic spacer sequence in this region. Such short intergenic regions were also found in other metazoans (Yatawara et al. 2010; Masta 2010; Yang and Yang 2008). Furthermore, previous studies have shown that the intergenic regions in the mt genomes of some metazoan contain signals for transcription initiation and replication (Cantatore and Attardi 1980; Goddard and Wolstenholme 1980). In the spacer region (*tRNA-Ser^(UCR)*-*nad1*), Tan et al. found a 6-bp motif (TACTTA), which is conserved between *A. cerana* and *A. mellifera*. This intergenic spacer region may correspond to the binding site of mtTERM, a transcription attenuation factor (Taanman 1999).

Table 8.4 Protein-coding gene (PCG) assignments and similarity between *A. cerana* and *A. mellifera*. (Tan et al. 2011)

Protein-coding gene	Number of amino acids		Identity (%)
	<i>A. cerana</i>	<i>A. mellifera</i>	<i>A. cerana/A. mellifera</i>
<i>atp8</i>	53	52	73.1
<i>atp6</i>	225	226	89.4
<i>nad1</i>	304	305	88.2
<i>nad2</i>	331	333	70.1
<i>nad3</i>	117	117	82.1
<i>nad4</i>	439	447	82.7
<i>nad4L</i>	87	87	85.1
<i>nad5</i>	556	554	82.7
<i>nad6</i>	169	167	51.5
<i>cox1</i>	521	521	97.5
<i>cox2</i>	226	225	95.6
<i>cox3</i>	259	259	78.8
<i>cob</i>	382	383	91.9

8.3 Protein-Coding Genes and Codon Usage Patterns

The boundaries between PCGs in the mt genome of *A. cerana* were determined by aligning their sequences and by identifying translation initiation and termination codons with comparison to those of *A. mellifera*. The amino acid sequences inferred for *nad3*, *nad4L*, *cox1* and *cox3* genes are similar in length to those of *A. mellifera* (Table 8.4). The similarity of the amino acid sequences is 51.5–97.5 % between *A. cerana* and *A. mellifera* (Table 8.4). Based on similarity, *cox1* is the most conserved PCG, while the *nad6* is the least conserved. Genes in mt genome may have different evolutionary rates, which might be caused by different selection pressures or the restriction of gene function. That is what Tan et al. (2011) found in honeybee that rates of different mitochondrial genes diverged differently.

The predicted translation initiation and termination codons for the PCGs of *A. cerana* mt genome were compared with those of *A. mellifera*. All protein-coding sequences started with a typical ATN codon. The start codons inferred in the mt genome of *A. cerana* are ATC (1 of 13 PCGs), ATT (8 of 13 PCGs), ATG (3 of 13 PCGs) and ATA (1 of 13 PCGs) as a translation initiation codon (Table 8.2). Furthermore, the anomalous initiation codon and incomplete stop codon frequently occur in PCGs of most of the Hymenoptera mtDNA sequenced to date, such as the gene *cox1* has been found to employ TTG as a start codon and *nad4* has been found to employ TA as a stop codon in *Vanhornia eucnemidarum* (Castro et al. 2006). The codon TGA, which is a termination codon in the universal code, codes for tryptophan in most mt genomes except those of higher plants (Yatawara et al. 2008). All stop codons used TAA as a termination codon in the mt genome of *A. cerana*. This is presumably polyadenylation after transcription to complete the termination codon (Ojala et al. 1981). However, 2 of the 13 PCGs (*cox1*, *cox2*) are predicted to employ T as an initiation codon in the mt genome of *A. mellifera* (Crozier and

Crozier 1993). Furthermore, incomplete stop codon frequently occurs in PCGs of most of the Hymenoptera mtDNA sequenced to date (Castro et al. 2006; Cha et al. 2007; Wei et al. 2010b).

The pattern of codon usage in the *A. cerana* mtDNA was also studied. Excluding the termination codons, a total of 3,672 amino acids are encoded by the *A. cerana* mt genome, and a total of 3,676 amino acids for *A. mellifera*. The most frequently used amino acid was Leu (15.52 %), followed by Ile (12.85 %), Phe (10.10 %), Ser (9.15 %) and Met (8.96 %) (Table 8.5).

8.4 Transfer RNA Genes

The 22 tRNA genes encoded in the mt genome of the *A. cerana* vary in length from 60 to 78 nucleotides with differences in stem and loop sizes of dihydrouridine (D) and TΨC loops. The order and orientation of the gene arrangement pattern are identical to that of *A. mellifera*, except for the position of the *tRNA-Ser^(AGN)* gene. All of the 22 tRNA genes have the typical cloverleaf structure, except for *tRNA-Ser^(AGN)* that lacks DHU arm (Fig. 8.1). Their putative secondary structures (Fig. 8.1) are similar to those of *A. mellifera* (Crozier and Crozier 1993; Cha et al. 2007; Silvestre et al. 2008), indicating their similar functions. Among Hymenoptera, mismatched base pairs have been reported in mitochondrial tRNAs inferred for *A. mellifera*, *Melipona bicolor*, *Diadegma semiclausum*, *Bombus ignitus* and *Evania appendigaster* (Crozier and Crozier 1993). A total of six mismatched base pairs occur in tRNAs of *A. cerana*. Two tRNA genes, *tRNA-Ala* and *tRNA-Gln*, have a single G-T and T-T mismatches in the acceptor stem, respectively. *tRNA-Val* has a single T-T mismatch in the anticodon arm, and *tRNA-Cys*, *tRNA-His* and *tRNA-Pro* all have a single G-T mismatches in the DHU arm. Sequences of three tRNAs overlap with adjacent genes, *tRNA-Cys* overlaps with the adjacent gene *nad2* on the opposite strand for 1 bp at its 3'-end. *tRNA-Leu^(UUR)* overlaps with the adjacent gene *cox1* on the same strand for 5 bp at its 3'-end. *tRNA-Asp* overlaps with the adjacent gene *cox2* on the same strand for 2 bp at its 3'-end. Finally, and most importantly, stem mismatches and sequence overlap are not uncommon for mt tRNAs of hymenopteran, and are probably repaired by a post-transcriptional editing process (Lavrov et al. 2000; Masta 2000). The 22 tRNA genes are located on both strands, 14 tRNAs are encoded on the H-strand and 8 on the L-strand (Table 8.2). In these tRNAs, there is a strict conservation of the sizes of the amino acid acceptor stem (13–15 bp) and the anticodon loop (7–9 bp). Their D-loops consist of 4–10 bp. The extra-variable loops of 4–5 bp and the TΨC loops of 4–10 bp complete the cloverleaf structures. In most metazoan mitochondria, *tRNA-Ser^(AGN)* generally has an unpaired DHU arm, while *tRNA-Ser^(UCN)* has a standard cloverleaf structure (Nakao et al. 2002, 2007). The *Apis tRNA-Ser^(AGN)* and *tRNA-Ser^(UCN)* structures conform to this interpretation.

Table 8.5 Codon usage in 13 protein-coding genes (PCGs) of the *Apis cerana* mitochondrial genome. (Tan et al. 2011)

Aa	Codon	N	RSCU	Aa	Codon	N	RSCU	Aa	Codon	N	RSCU	Aa	Codon	N	RSCU
Phe	UUU	336	1.81	Ser	UCU	55	1.31	Tyr	UAU	202	1.84	Cys	UGU	26	2.00
	UUC	35	0.19		UCC	9	0.21		UAC	17	0.16		UGC	0	0
Leu	UUA	483	5.08	Pro	UCA	167	3.98	End	UAA	0	0	Trp	UGA	84	2.00
	UUG	10	0.11		UCG	0	0		UAG	0	0		UGG	0	0
Leu	CUU	40	0.42	Thr	CCU	34	1.30	His	CAU	55	1.80	Arg	CGU	9	0.95
	CUC	1	0.01		CCC	4	0.15		CAC	6	0.20		CGC	1	0.11
Ile	CUA	34	0.36	Gln	CCA	67	2.55	Asn	CAA	39	2.00	Ser	CGA	28	2.95
	CUG	2	0.02		CCG	0	0		CAG	0	0		CGG	0	0
Met	AUU	452	1.92	Ala	ACU	38	1.26	Lys	AAU	223	1.88	Gly	AGU	15	0.36
	AUC	20	0.08		ACC	1	0.03		AAC	14	0.12		AGC	0	0
Val	AUA	312	1.90	Glu	ACA	82	2.71	Asp	AAA	156	1.91	Gly	AGA	85	2.02
	AUG	17	0.10		ACG	0	0		AAG	7	0.09		AGG	5	0.12
Val	GUU	83	2.21	Glu	GCU	29	1.63	Glu	GAU	60	1.94	Gly	GGU	44	1.30
	GUC	7	0.19		GCC	4	0.23		GAC	2	0.06		GGC	3	0.09
Val	GUA	59	1.57	Glu	GCA	36	2.03	Glu	GAA	77	1.86	Gly	GGA	82	2.43
	GUG	1	0.03		GCG	2	0.11		GAG	6	0.14		GGG	6	0.18

A total of 3,672 codons for *A. cerana* were analysed, excluding the termination codons *Aa* amino acid, *N* frequency of each codon, *RSCU* relative synonymous codon usage

8.5 Ribosomal RNA Genes

The *rrnS* and *rrnL* genes of *A. cerana* were identified by sequence comparison with those of *A. mellifera*. The *rrnS* is located between *tRNA-Leu*^(CUN) and *tRNA-Val*, and the *rrnL* is located between *tRNA-Val* and the A + T-rich region. The length of the *rrnS* and *rrnL* genes of *A. cerana* is 786 bp and 1,328 bp, respectively, but is relatively shorter than the genes in *A. mellifera* (827 bp and 1,371 bp, respectively) (Table 8.2). The A + T contents of the *rrnS* and *rrnL* for *A. cerana* are 81.6 % and 83.1 %, respectively (Table 8.3). Sequence identity in the *rrnS* and *rrnL* genes is 53.9 % and 86.0 % between *A. cerana* and *A. mellifera*, respectively.

8.6 A + T-Rich Region

The A + T-rich region is believed to be involved in the regulation of transcription and control of DNA replication, characterized by five elements: (1) a polyT stretch at the 5'-end of the A + T-rich region, which may be involved in the control of transcription and/or replication initiation; (2) a [TA(A)]_n-like stretch following the polyT stretch; (3) a stem and loop structure, which may be associated with the second strand-replication origin; (4) a TATA motif and a G (A)_nT motif flanking the stem and loop structure and (5) a G + A rich sequence downstream of the stem and loop structure (Zhang and Hewitt 1997). The A + T-rich region is well known for the initiation of replication in both vertebrates and invertebrates, and the reduced G + C content is one of the most outstanding features of this region (Boore 1999).

The largest non-coding region (562 bp) in the *A. cerana* mt genome is flanked by *rrnS* and *tRNA-Ser*^(AGN) and it is highly enriched in AT (95.5 %). In this region, Tan et al. (2011) found some of the elements commonly present in most insects' A + T-rich region, such as polyT stretch, [TA(A)]_n-like stretch, stable stem-loop secondary structures (not shown), and TATA motif, which may function in the initiation of genome replication. Based on these features, it possibly functions as a control region. The AT-region of *Diadegma semiclausum* has the highest percentage of A + T content (96.4 %) and *Orussus occidentalis* has the lowest A + T content (79.7 %) among Hymenoptera sequenced to date (Table 8.6).

8.7 Phylogenetic Analyses

Many systematic and population genetic studies have been based on genetic markers in the mt genomes at both the nucleotide and amino acid levels (Rand and Kann 1998; Tourasse and Li 2000). So far, three mt genes (*rrnL*, *cox2*, *nad2*) and two nuclear genes (EF1- α intron and *itpr*) have been used for phylogenetic study of members within the genus *Apis*, yielding discrepant results (Yudin and Crozier 2007; Arias and Sheppard 2005). Usage of complete mt sequences for phylogenetic analyses would be more reliable, but so far only two complete mt genomes (*A. cerana* and *A. mellifera*) are available within the genus *Apis*. To better understand the evolution of

Table 8.6 Comparison of A + T content (%) of the AT region, protein-coding and rRNA genes of mitochondrial genomes of Hymenoptera. (Tan et al. 2011)

Gene/region	AE	AC	AM	BH	BI	CC	CV	DS	ESP	EA	MB	OO	PSP	PH	SA	VE
AT-region	89.9	95.9	96.0	/	96.0	88.3	/	96.4	88.1	85.6	/	79.7	78.9	/	/	/
<i>atp8</i>	86.1	87.0	89.3	92.6	92.6	82.8	93.0	89.5	91.7	69.1	91.1	82.4	86.5	93.7	90.1	82.7
<i>atp6</i>	80.4	83.8	84.7	85.0	85.4	77.3	86.4	84.1	84.6	73.9	86.6	74.8	77.6	83.1	84.3	76.3
<i>nad1</i>	80.6	83.5	83.0	82.7	84.7	79.8	85.3	85.2	82.3	74.6	86.4	75.0	75.4	84.7	79.0	79.0
<i>nad2</i>	80.9	86.4	86.5	89.0	89.8	85.8	91.2	89.2	88.9	80.5	91.2	75.3	80.4	89.9	88.8	83.7
<i>nad3</i>	80.5	84.8	86.4	88.3	90.0	78.3	88.7	85.0	87.3	74.1	89.0	75.0	78.7	84.5	83.5	79.9
<i>nad4</i>	82.1	85.1	86.5	85.5	85.9	81.3	87.5	85.8	86.0	77.8	89.2	77.0	77.4	84.1	83.7	80.1
<i>nad4L</i>	83.5	87.1	85.6	86.1	88.0	85.0	88.7	89.1	88.1	77.5	90.7	82.5	81.3	89.1	87.6	85.0
<i>nad5</i>	80.7	84.9	85.7	86.9	87.4	80.5	88.8	86.0	86.3	76.3	88.6	77.4	77.9	85.7	84.2	80.6
<i>nad6</i>	84.5	86.5	86.9	92.3	92.1	85.1	92.3	90.2	90.1	78.7	93.0	77.3	82.5	90.3	88.8	82.7
<i>rns</i>	80.4	81.6	81.4	85.4	86.6	80.5	91.2	88.5	89.4	75.8	82.8	81.1	77.1	84.0	89.3	80.8
<i>rnl</i>	82.9	83.1	84.5	85.3	85.5	84.6	89.7	88.0	87.2	79.7	86.8	80.1	79.4	87.3	88.8	82.9
<i>cox1</i>	72.0	76.0	75.9	76.4	71.7	77.6	72.7	77.2	76.1	77.6	79.2	68.8	75.3	70.3	75.3	75.8
<i>cox2</i>	75.5	78.9	80.5	81.0	82.0	74.9	83.3	81.3	82.6	72.0	83.0	72.1	73.7	80.5	80.4	74.0
<i>cox3</i>	75.4	80.4	83.0	82.6	84.8	75.6	83.6	80.2	80.2	73.9	84.7	72.1	72.3	80.5	77.2	77.0
<i>cob</i>	75.6	81.0	80.6	81.0	82.8	77.0	82.3	80.1	81.0	73.3	82.6	71.1	73.0	81.2	78.9	75.7
EmtG	80.6	84.0	84.9	85.3	86.8	82.0	87.2	87.4	85.2	77.8	86.7	76.2	77.0	84.7	84.0	80.1

AE *Abispa ephippium*, AC *Apis cerana*, AM *Apis mellifera*, BH *Bombus hypocrita*, BI *Bombus ignitus*, CC *Cephus cinctus*, CV *Cotesia vestalis*, DS *Diadegma semiclausum*, ESP *Enicospilus* sp., EA *Evania appendigaster*, MB *Melipona bicolor*, OO *Orussus occidentalis*, PSP *Philaenus spumarius*, PH *Polistes humilis*, SA *Spathius agrili*, VE *Vanhornia eucnemidarum*, EmtG entire mitochondrial genome

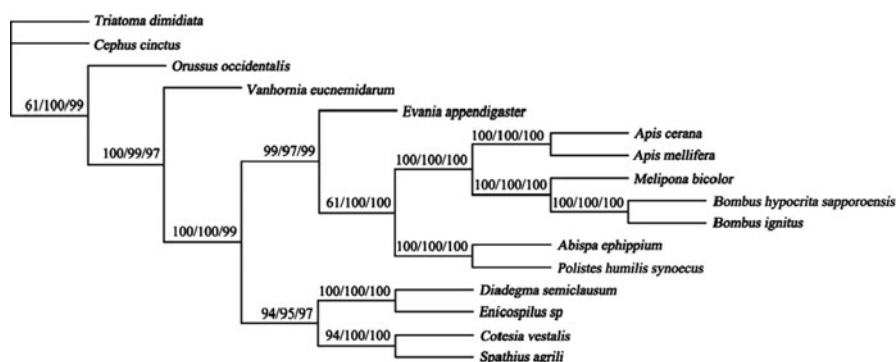


Fig. 8.3 Inferred phylogenetic relationship among the Hymenoptera species. The analyses were conducted using maximum parsimony (MP), maximum likelihood (ML) and neighbour joining (NJ) of amino acid sequences of 13 protein-coding genes (PCGs), using *Triatoma dimidiata* as out-group. The numbers along branches indicate bootstrap values resulting from different analyses in the order: MP/ML/NJ. (Tan et al. 2011)

genome-level features in *A. cerana*, phylogenetic relationships among representative members of the Hymenoptera were inferred from concatenated amino acid sequences of the 13 mt PCGs. With addition of the *A. cerana* mt genome sequences, there are now 28 hymenopteran sequences available for analysis, and only 16 of these have the complete sequences of the 13 mt PCGs. The final alignment of the amino acid sequences of 13 PCGs for the 16 taxa were subjected to the ML, MP and NJ analyses. The results revealed that *A. cerana* and *A. mellifera* are closely related with high statistical support, indicating that *A. cerana* and *A. mellifera* have a sister relationship (Fig. 8.3), supporting the taxonomic classification by the analyses of morphological and molecular data.

Three major clades were recovered within Apidae: Apini (*A. cerana* and *A. mellifera*), Meliponini (*M. bicolor*) and Bombini (*B. ignites* + *B. hypocrita sapporoensis*) that were strongly supported in all (ML, MP and NJ) analyses (Tan et al. 2011). These results were congruent with previous studies (Dowton et al. 2009b; Wei et al. 2010b). Our data provide a robust support for the relationship among Hymenoptera.

In conclusion, the mt genome of *A. cerana* exhibits almost the same characteristics with that of *A. mellifera*. Phylogenetic analyses indicated that *A. cerana* and *A. mellifera* are two separate species, supporting the taxonomic classification by genetic relatedness and phylogeny. The analyses of *A. cerana* mt genome have added to our knowledge on mt genomes of Hymenoptera, and provided additional genetic markers for studying population genetics, systematics and phylogeographics of honeybees.

8.8 Amplification and Sequencing of Partial *cox1*, *nad4* and *rrnL*

Partial fragments of the *cox1*, *nad4* and *rrnL* mtDNA genes were amplified using three sets of primers (Table 8.7), designed by the authors according to homological mtDNA fragments of other hymenopterans deposited in GenBank (Tan et al. 2011).

PCR reactions were carried out in a 25 μ l reaction volume consisting of 14.25 μ l sterile deionized water, 2.5 μ l 10 $\times$ PCR buffer (Mg^{2+} free), 4.0 μ l MgCl_2 (25 mM), 2.0 μ l dNTPs (2.5 mM each), 0.25 μ l each primer (50 pmol/ μ l), 0.25 μ l ExTaq DNA polymerase (5 U/ μ l, Takara) and 1.5 μ l DNA template (40 ng/ μ l) with the following conditions: after an initial denaturation at 94 °C for 5 min, then 94 °C for 30 s (denaturation), 45–50 °C for 30 s (annealing), 72 °C for 30 s (extension) for 35 cycles, followed by 72 °C for 5 min (final extension). Each amplicon (5 μ l) was examined by agarose gel electrophoresis to validate amplification efficiency. Then, the partial *cox1*, *nad4* and *rrnL* amplicons were sent to Takara Company (Dalian, China) for sequencing from both directions by using primers used in the PCR amplifications.

8.9 Long-PCR Amplification and Sequencing

The nucleotide sequences obtained from these three genes were then used to design *A. cerana*-specific primer sets for long-PCR reactions. Three overlapping long-PCR fragments covering the entire mt genome of *A. cerana* were obtained using the long-PCR primers sets (Table 8.7). The long-PCR reaction volume amounted 50 μ l containing 28.5 μ l sterile deionized water, 5.0 μ l 10 $\times$ LA PCR buffer (Mg^{2+} free), 5.0 μ l MgCl_2 (25 mM), 8.0 μ l dNTPs (2.5 mM each), 0.5 μ l each primer (50 pmol/ml), 0.5 μ l LA Taq DNA polymerase (5 U/ μ l, Takara) and 2 μ l DNA template (40 ng/ml). Long-PCR cycling conditions used were initial denaturation step at 92 °C for 2 min, followed by 30 cycles of denaturation at 92 °C for 10 s, primer annealing at 50 °C for 30 s and elongation at 60 °C for 10 min during the first 10 cycles, and then an additional 10 s per cycle during the last 20 cycles. The final elongation step was continued at 60 °C for 10 min. All amplifications were done on a T-Gradient thermocycler (Biometra, Germany). The three long-PCR fragments were sequenced using a primer-walking strategy. All sequencing was performed with ABI PRISM Big Dye terminator chemistry and analysed on ABI 3730 automated sequencers (Applied Biosystems, USA).

8.10 Gene Annotation and Sequence Analysis

Sequences were assembled manually and aligned against the complete mt genome sequence of *A. mellifera* using the computer program ClustalX 1.83 (Thompson et al. 1997) to identify gene boundaries. The open-reading frames and codon usage profiles of PCGs were analysed using the program MacVector 4.1.4 (Kodak, version 4.0). Translation initiation and translation termination codons were identified based on comparison with the mt genome of *A. mellifera*. The amino acid sequences inferred for the mt genes of the *A. cerana* were aligned with those of *A. mellifera* by using ClustalX 1.83. Based on pair-wise alignments, amino acid identity (%)

Table 8.7 Sequences of primers used to amplify PCR fragments from the *Apis cerana* mitochondrial genome. (Tan et al. 2011)

Primers	Sequence (5'–3')	Estimated size of PCR products
AC- <i>cox1</i> F	ATTAGATTCTGATTGCTTCCT	680 bp
AC- <i>cox1</i> R	TATGTTGCTAATCATCTAAAT	
AC- <i>nad4</i> F	ATTAATCGTCTATTAGTTTG	
AC- <i>nad4</i> R	TAAAAGCTCATGTTGAAGC	370 bp
AC- <i>rrn</i> LF	TGAACTCAAATCATGTAAGAT	
AC- <i>rrn</i> LR	ACTGTACAAAGGTAGCATAAT	
AC- <i>Lcox1</i> F	AGAAATTTATTTTATCCAAGACCAGGAACAG	7.1 kb
AC- <i>Lnad4</i> R	TGAAATTAGGAGGTTATGGGATATTACGTT	
AC- <i>Lnad4</i> F	AACATTAACATAAAAAAATAAACCTGAAGAT	
AC- <i>Lrrn</i> LR	CCATGAAATAAATTTAAATAGCTGCAGTA	4.8 kb
AC- <i>Lrrn</i> LF	TGCGTTTAACTTTTCTCTTAATTCAACATC	
AC- <i>Lcox1</i> R	TTGCTGAAGTAAATAAGCTCGTGATCAA	

was calculated for homologous genes. Codon usage was examined based on the relationships between the nucleotide composition of codon families and amino acid occurrence, where the genetic codons are partitioned into AT-rich codons, GC-rich codons and unbiased codons. For analysing rRNA genes, putative secondary structures of 22 tRNA genes were identified using tRNAscan-SE (Lowe and Eddy 1997), of the 22 tRNA genes, 19 were identified using tRNAscan-SE, the other 3 tRNA genes were found by eye inspection, and rRNA genes were identified by comparison with the mt genome of *A. mellifera*.

Phylogenetic relationships among Hymenoptera were identified using the 14 Hymenoptera species (Table 8.1) as in-group, plus the mtDNA sequence of *A. cerana* obtained in the study by Tan et al. (2011), using one Reduviidae species (*Triatoma dimidiata*, GenBank accession number AF301594) as the out-group, based on amino acid sequences of 13 PCGs. Amino acid sequences for each gene were individually aligned using ClustalX 1.83 (Thompson et al. 1997) under default setting, and then concatenated into single alignments for phylogenetic analyses. Three methods, namely neighbour joining (NJ), maximum likelihood (ML) and maximum parsimony (MP), were used for phylogenetic reconstructions. Standard unweighted MP was performed using package Phylip 3.67 (Felsenstein 1995). NJ analysis was carried out using PAUP 4.0 Beta 10 program (Swofford 2002), and ML analysis was performed using PUZZLE 4.1 under the default setting (Strimmer and Haeseler 1996). The consensus tree was obtained after bootstrap analysis, with 1,000 replications for NJ and MP trees, and 100 for ML tree, with values above 50 % reported.

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Chapter 9

Interactions with Other Bee Species

9.1 Introduction

The Asian continent is the richest in the world in honeybee diversity and includes a number of indigenous species: *Apis cerana*, *Apis florea*, *Apis andreniformis*, *Apis dorsata*, *Apis laboriosa*, *Apis nigrocincta*, *Apis nuluensis* and *Apis koschevnikovi* as well as the introduced *Apis mellifera* which is widely used for honey production. When these *Apis* species occur sympatrically, they can interact in various ways (Koeniger 1982). Worker bees of different species may rob each others' nests and compete for food or for nesting sites, while drones may interfere with each other during mating flights. Besides, a parasite or disease of one species may transfer to another which it is not resistant to the parasite or disease. Interspecific interactions among the *Apis* species have no doubt played a role in their evolution. Even though interspecific interactions of the present may not be like those of the past, before or during the process of speciation, it is still an interesting and potentially important topic that deserves investigation. Since the male genitalia which are regarded as one of the most important factors in reproductive isolation and speciation among some species are not completely distinct, the possibilities of food and/or nest competition might make more sense in considering speciation in the genus *Apis*.

9.2 Nest Site Competition

In Asia, the honeybee species have adopted different evolutionary strategies to adapt to their environments and, according to body size and nesting habits, they can be divided into three groups: dwarf honeybees, giant honeybees, and cavity-nesting honeybees (Arias and Sheppard 2005; Oldroyd and Wongsiri 2006). Given that each of them has a distinct nesting behavior, nest site competition between them can rarely be observed, so in this section only competition within each group is discussed.

9.2.1 Nest Site Competition in the Dwarf Honeybees

The dwarf honeybees include two species, *A. florea* and *A. andreniformis*, and both naturally occur in tropical and sub-tropical regions of Asia (Wongsiri et al. 1996). *A. florea* extends from the Middle East eastwards to peninsular Malaysia, whereas *A. andreniformis* is distributed from the Philippines to China and Myanmar, but they overlap in Southeast Asia (Otis 1996; Wongsiri et al. 1996; Hepburn and Radloff 2009). So our interest lies in whether they compete for nest sites in the limited areas where they overlap.

These two honeybee species are superficially similar in many respects and it took a number of years for honeybee biologists to define them as unequivocally separate species (Smith 1858; Maa 1953; Wu and Kuang 1987; Ruttner 1988; Wongsiri et al. 1990; Hepburn et al. 2005). As for nest sites, both species build single, exposed combs on the thin branches of bushes, shrubs or small trees (Wongsiri et al. 1996) and, in western Asia, often nest in small caves or in sheltered areas of buildings (Dutton and Free 1979; Whitcomb 1984). Although it has been reported that the two species may also nest at different altitudes: *A. andreniformis* in high mountainous and forest areas at about 1,600 m altitude, while *A. florea* is common in lowlands below 1,000 m (Wongsiri et al. 1996), an analysis of the complete distribution of the species shows that there is no significant difference in their altitudinal distributions (Hepburn and Radloff 2009).

However, the nests of *A. andreniformis* appear higher (about 6 m from the ground) than those of *A. florea* (about 4 m) (Rinderer et al. 2002), so that nesting competition between them can be inferred to happen only occasionally. In addition, Rinderer et al. (2002) did find that when these two species occur together in the same area, they tend to avoid each other. Such avoidance between these two species, although still controversial, may make sense if the two species evolved the ability to recognize each other during the course of speciation and mutual adaptation. Interestingly, Rinderer et al. (2002) reported that both species of dwarf honeybees have a tendency to form aggregations of colonies in spatial distribution, but not as intensely as colonies of *A. dorsata*.

9.2.2 Nest Competition in the Giant Honeybees

Two species, *A. dorsata* and *A. laboriosa*, form the group *MegApis*, or giant honeybees. *A. dorsata* is distributed mainly in tropical areas while *A. laboriosa* naturally occurs in mountainous regions, particularly the Himalayas, at altitudes between 1,500 and 4,000 m (Sakagami et al. 1980; Ruttner 1988; Underwood 1990a, b). The former species has a tendency to be highly aggregated, 100 or even more colonies crammed onto a single tree (Deodikar et al. 1977; Seeley et al. 1982; Dyer and Seeley 1991), and has a habit of seasonal migration. The latter also has a tendency for colony aggregations but only on cliffs (Roubik et al. 1985; Kuang and Kuang 2002; Joshi et al. 2004). According to Underwood (1990a, b), *A. laboriosa* never nests on the

limbs of trees. Although they also have seasonal migration behavior, which results in a temporary sympatry with *A. dorsata*, it occurs during the non-nesting phase of *A. laboriosa*. So, we can safely conclude that nest competition between these two giant honeybees does not occur at present and can only speculate as to the past.

9.2.3 Nest Competition in the Cavity-Nesting Honeybees

Cavity-nesting honeybees include *A. mellifera*, *A. cerana*, *A. nigrocincta*, *A. nuluensis*, and *A. koschevnikovi*, all of which are native to Southeast Asia except *A. mellifera*. The four Asian cavity-nesting honeybees began their divergence from a presumed cosmopolitan *A. cerana* prototype some 2 million years ago (Smith 1991; Arias and Sheppard 2005). Even so, the habitats of each species are very different. For example, *A. nuluensis* is confined to the highlands on the island of Borneo (Malaysia), and it is only known from the Crocker Range in Sabah (Tingek et al. 1996). The Sulawesi honeybee, *A. nigrocincta*, is confined to the islands of Sulawesi, Sagihe, and Mindanao (Otis 1996). *A. koschevnikovi* has a comparatively wider distribution area: from Java, Sumatra, peninsular Malaysia to southern Thailand, however, since this bee requires rainforest habitat, it is now rare outside of Borneo owing to deforestation (Hadisoesilo et al. 2008). *A. nuluensis* is confined to mountainous regions above 1,500 m only on the spectacular Mount Kinabalu, in the Malaysian state of Sabah in Borneo (Tingek et al. 1996). *A. cerana* occurs on the mainland of Asia as well as the islands of the South China Sea (Radloff et al. 2009). For these combined reasons, we have not seen many reports about nesting competition among these cavity-nesting honeybees. Interestingly, all these cavity-nesting honeybees, except *A. cerana*, mainly occur on islands in the South China Sea, the islands providing perfect geographic isolation, which undoubtedly has played a very important role in the speciation of these honeybees. It seems that the only practical place to investigate possible nest competition among these species is Borneo, where three cavity-nesting bees: *A. koschevnikovi*, *A. nuluensis* and *A. cerana* coexist or Sulawesi where *A. cerana* and *A. nigrocincta* are sympatric.

9.2.4 Social Parasitism

Social parasitism in honeybees is generally understood to mean the phenomenon of worker bees joining neighboring colonies by drifting or direct invasion (Neumann et al. 2001a). Social parasitism is widespread in social insects but has been studied only in *A. cerana* and *A. florea* amongst Asian honeybees. Nanork et al. (2006a) found that in queenright *A. cerana* colonies, 2–6 % of workers are non-natal, but these drifted workers do not have active ovaries, suggesting that in queenright colonies social parasitism is not pervasive. However, in queenless colonies, there were significantly more non-natal workers (72.7 %) with activated ovaries than natal workers

(36.3 %). Non-natal workers also had a significantly higher reproductive success than natal workers. The same phenomenon has been observed in the dwarf honeybees, *A. florea* (Nanork et al. 2006a; Chapman et al. 2009). In *A. florea* colonies, when a colony becomes queenless, workers bees have a higher tendency for parasitizing other colonies, preferring queenless to queenright colonies as their hosts for reproduction; and, as a result, queenless colonies are heavily parasitized with the eggs of non-natal workers (Nanork et al. 2006b). It has been suggested that social parasitism is present more or less in all honeybees species: 2–4 % of the workers are non-natal, although these unrelated workers are thought to arise via orientation errors while returning from foraging trips (Chapman et al. 2009).

Although social parasitism has only been observed intraspecifically in honeybees, interspecific parasitism has yet to be investigated. However, *A. cerana* was observed in a colony of *A. mellifera* for a short period but subsequently flew away (Denis Anderson, personal communication) and *A. cerana* workers have been seen on nests of *A. florea* (Duangphakdee, Hepburn, Phiancharoen, personal communication). The same phenomenon has been reported in *A. mellifera capensis* invading colonies of *A. m. scutellata* by Neumann et al. (2001b). During the long history of evolution, parasitism might have played a role in nest competition and/or nest avoidance in speciation.

9.3 Food Competition

Besides possible competition for habitat and reproduction, the species also compete for food resources when they occur in the same area. The performance of different bees in competition is of significance in speciation and/or coevolution. When different honeybees compete for food, body size is an important factor and the smaller bees are usually more aggressive in defending floral resources, probably because smaller bees have more restricted foraging ranges than the larger ones (Ruttner 1988).

Koeniger and Vorwohl (1979) investigated the interactions of three honeybee species: *A. florea*, *A. cerana*, and *A. dorsata* and stingless bees *Trigona* by using an artificial feeding dish. They found that small bees generally attacked larger ones, but, *A. dorsata* was attacked only by *A. cerana*, never by the other two species. At times, only one species remained while the others stayed away, but a final winner was unpredictable. Ruttner (1988) concluded that honeybees with larger bodies enjoy more choices, usually avoid disastrous fighting and shift to other, more distant food resources.

In Nepal, Partap (1998) investigated the impact of the introduction of *A. mellifera* colonies on the foraging behavior of a local honeybee, *A. cerana*. Foraging competition was studied by counting the number of foragers of *A. cerana* on several flowers during the presence of and after removal of *A. mellifera* colonies (Table 9.1). The results indicated that *A. cerana* foragers spend more time visiting flowers in the absence of *A. mellifera*. They also spend more time on flowers, visit more flowers per trip, collect more pollen, and more *A. cerana* foragers were seen on the flowers when the competition from *A. mellifera* was removed.

Table 9.1 Mean number ($\pm$ S.D.) of *A. cerana* foragers during the presence of and after removal of *A. mellifera*. (Partap 1998)

Crop	Number of <i>A. cerana</i> foragers		Difference significance
	During the presence of <i>A. mellifera</i>	After the removal of <i>A. mellifera</i>	
Mustard	12.6 $\pm$ 1.2	20.8 $\pm$ 1.3	$p < 0.01$
Broadleaf mustard	12.3 $\pm$ 1.3	18.3 $\pm$ 2.1	$p < 0.01$
Cauliflower	18.4 $\pm$ 1.1	28.3 $\pm$ 0.8	$p < 0.01$
Radish	11.7 $\pm$ 0.9	16.2 $\pm$ 1.2	$p < 0.01$

Similarly, Dhaliwal and Atwal (1970) studied food competition between *A. cerana indica* and *A. mellifera* at feeding dishes. Firstly, the two species were fed at their own respective feeders, not mixing with each other, and showing no hostile behavior, but as the feeders were brought nearer to each other, the bees became more and more aggressive. When *A. mellifera* workers were freely alighting on both feeders, *A. cerana* workers were hesitant to do so, and the latter were often stung by the former, some dying, but no *A. mellifera* died. Finally, *A. mellifera* workers formed a ring around the feeder while *A. cerana* workers could not alight to feed. The results indicated that *A. mellifera* was more successful in eliminating *A. cerana*. This suggests that honeybees can distinguish their nestmates outside of the hive, and can jointly compete for food. Kalmus (1941) found that even different strains of the same species can distinguish each other. Two colonies of differently colored *A. mellifera* bees, Caucasians and Italians, were trained to feeders and behaved aggressively towards each other. So we can infer that during speciation, the newly forming species could probably recognize their own nestmates and fight others, which in turn could be expected to facilitate speciation. Stout and Goulson (2001) found that bumblebees (*Bombus* spp.) and honeybees (*A. mellifera*) are both able to use odor cues deposited on flowers by previous visitors. Both bumblebees and honeybees avoided flowers previously visited by each other when foraging on *Melilotus officinalis*, that is, bumblebees avoided flowers recently visited by honeybees and vice versa.

How do honeybees avoid serious competition among different species? Different species have different strategies which shape their foraging decisions such as energy consumption and body temperature of foragers. Firstly, body size and the length of the proboscis surely play an important role in competition, and the size of a forager may determine which floral resources are available to it. For example, *A. dorsata*, one of the giant honeybees, can fly farther than smaller honeybees and so enjoy larger foraging areas. As they have a longer glossa, they may be able to collect nectar from some deep flower corolla tubes, but not able to gain access to some very small flowers with deeply hidden nectaries. Evidently in the course of coevolution, flowers of highly specialized morphology have developed nectaries for specific pollinators, and foragers of the different species specialize on particular floral resources (Oldroyd and Wongsiri 2006). Secondly, different species have different flight designs. Some researchers have intensively investigated the flight designs of honeybees (Hepburn et al. 1998a, b; Hepburn et al. 1999; Radloff et al. 2003), and have suggested that several factors can integrate into an excess power index (EPI) that determines the

flight ability of honeybees. These factors include: whole body mass, thoracic mass, thorax/body mass ratio, wing surface area, and wing loading. The EPI is defined as (r^2/W) where W is the wing loading and r is the ratio of the thorax mass to total mass (Hepburn et al. 1998b). According to this index, the drones of Asian honeybees can be statistically divided into two groups: dwarf honeybee drones form one group and the other species belong to the other group. As for workers, the EPI can divide the Asian honeybees into three groups. It is suggested that prowess of flight in drones is driven by the need to compete and mate with queens flying high in the air while worker bees forage nectar and pollen on flowers (Radloff et al. 2003).

Dyer and Seeley (1991) reported that among Asian species, *A. cerana* show a disproportionately high mass-specific metabolic rate, their foragers make many more trips per day in the same habitat than do foragers of the other species. Last but not least, different species differ in both the times and temperatures to initiate their collecting trips. *A. cerana* colonies start their work earlier in the day than *A. mellifera* workers and can endure lower ambient temperatures and are more industrious in collecting nectar from scattered flowers, while *A. mellifera* workers tend to prefer big flower patches (Kuang and Kuang 2002). Oldroyd et al. (1992) investigated foraging competition among four species: *A. dorsata*, *A. cerana*, *A. andreniformis*, and *A. florea* in Thailand on inflorescences of the king palm *Archontophoenix alexandrae*, which produces copious quantities of pollen overnight. Only the earliest visitors can collect the large amount of nectar available just before dawn. In order of appearance, *A. cerana* comes first, followed by *A. dorsata* shortly after dawn, and less than an hour later they are replaced by *A. florea* and *A. andreniformis* and some stingless bees.

This partitioning of resources depend upon two important factors: body temperature and energy consumption. Honeybee biologists have noticed that a thoracic temperature threshold is absolutely crucial for a forager to initiate a flight trip (Dyer and Seeley 1987). A forager can increase her thoracic temperature by producing metabolic heat if the colony temperature is lower than the ambient temperature. The cavity-nesting species have the advantage of maintaining a higher nest temperature, which explains why *A. cerana* foragers begin collecting before dawn and earlier than other open-nesting bees. Fighting and food searching are a high energy consumption tasks, and the bigger the body size, the more energy required. This may be the reason why the giant honeybees can fly further and exploit other flowers rather than fighting against the smaller bees.

9.4 Robbing

Robbing is an act, or a series of acts, by which bees from one colony rob, pilfer, or steal honey from other colonies (Ribbands 1953; Atwal and Dhaliwal 1969). This differs fundamentally from food competition, which happens outside the nests, because robber bees enter the nests of other colonies, kill bees, and take the stores. The smaller the colony the more susceptible it is to the loss of the stores and death of the workers (Hepburn and Radloff 1998). Usually every colony has some guard bees at the

entrance to fight against intruders, and these guard bees are able to distinguish their nestmates by their colony specific odors (Ribbands 1954, 1955). Robbing usually occurs in times of dearth when there is not enough available nectar (Hepburn and Radloff 1998). However, robbing may occur at any time when the nectar flow is interrupted or the colonies become weak or diseased (Atwal and Dhaliwal 1969). Atwal and Dhaliwal (1969) investigated robbing behavior between *A. cerana indica* and *A. mellifera* and found that *A. cerana indica* bees are more prone to robbing than *A. mellifera*. However, Breed et al. (2007) suggested that robbing may be more characteristic of *A. mellifera* than other species. They compared nestmate recognition in several Asian honeybee species, *A. florea*, *A. andreniformis*, *A. dorsata* and *A. cerana*, and found that none of these species displayed strong aggressive responses to conspecific non-nestmates. This result indicates that *A. mellifera* has a more strongly developed response to conspecific non-nestmates than other *Apis* species. This conclusion explains what happens in China, when *A. cerana* and *A. mellifera* colonies are kept at the same apiaries. They rob each other during times of dearth, and it has been reported that *A. cerana* is more likely to initiate robbing, but they usually lose when *A. mellifera* robs back (Yang 2001). Numerous *A. cerana* colonies were killed in this way and lost territory in some areas (Yang 2001). Other interspecific instances of robbing between *A. florea* and *A. mellifera* were reported by Koeniger (1976a), and by Atwal and Dhaliwal (1969) between *A. dorsata* and *A. cerana indica*. Robbing by *A. dorsata* is most serious in *A. mellifera* colonies when the latter are located near their nesting sites. In one instance more than 100 colonies of *A. mellifera* were devastated by *A. dorsata*, the foragers of which stormed into the hives of *A. mellifera* (Abrol, unpublished data).

Robbing can also be a means of transmitting bee diseases and parasites, as shown by Atwal and Dhaliwal (1969) who reported that at a mixed apiary in India, under natural conditions, *A. mellifera* were free from acarine disease, but after robbing some weak *A. cerana* colonies, 70–80 % of two *A. mellifera* colonies were infested. They also found that the acarine mite could be transmitted under experimental conditions from diseased *A. cerana* colonies to healthy *A. mellifera* colonies. Although mites occur on several *Apis* species, (Koeniger et al. 1983; Delfinado-Baker et al. 1985; Kuang and Kuang 2002), interspecific transmission has seldom been reported, except the *Varroa* mites from *A. cerana* to *A. mellifera* (Crane 1990), and *Neocyphophthalmus* mites from *A. cerana* to *A. florea* and *A. dorsata* via foraging on the flowers (Koeniger et al. 1983).

9.5 Intervention of Mating

Among the most interesting of the interspecific interactions between the Asian *Apis* species are those arising from the numerous attempts to investigate the intervention of mating. As queens of all the honeybee species have similar queen pheromones by which drones locate the virgin queens, 9-oxo-2-decenoic acid, or commonly abbreviated as 9-ODA (Butler et al. 1967; Shearer et al. 1970; Koeniger and Wijayagunasekera 1976), drones from one species might fly after queens of another

Table 9.2 The key figures of reproduction in *Apis* species. (Woyke 1975; Ruttner 1988; Koeniger et al. 1996a; Koeniger et al. 1996d; Koeniger and Koeniger 2000; Baer 2005)

Species	Drone weight (mg) (mean $\pm$ S.D.)	Total number of sperm per drone ($\times 10^6$)	Queen weight (mg)	Sperm in spermatheca of queen ($\times 10^6$)	Sperm length (μ)	Mating frequency
<i>A. andreniformis</i>	70.8 $\pm$ 3.0	0.13	112	1.3	–	10.5
<i>A. florea</i>	77.6 $\pm$ 2.6	–	86	1.1	205.81	7.9
<i>A. cerana</i>	83.4 $\pm$ 8.9	1.0 $\pm$ 0.1	122	1.4	267.07	14.1
<i>A. koschevnikovi</i>	105.5 $\pm$ 5.6	1.7 $\pm$ 0.16	170	2.1	–	13.3
<i>A. nuluensis</i>	107.0 $\pm$ 6.7	1.3 $\pm$ 0.1	–	–	–	–
<i>A. nigrocincta</i>	–	–	–	–	–	40.3
<i>A. mellifera</i>	211.1 $\pm$ 11.8	12.7	202	4.7	262.69	11.6
<i>A. dorsata</i>	155.7 $\pm$ 8.5	–	290	3.9	218.69	44.2
<i>A. laboriosa</i>	–	–	–	–	–	28.4

species and try to mate with them. How queens avoid interspecific mating and therefore evolved into different honeybee species has long been a puzzle. Intervention of mating among the Asian honeybee species has been widely investigated, and it has been suggested that three factors can lead to mating isolation: differences in male genitalia (Koeniger and Koeniger 1991), different drone congregation areas (DCA) (Koeniger and Koeniger 2000), and different mating times (Koeniger and Koeniger 2000). All these factors are prezygotic barriers against interspecific mating, and if interspecific mating really occurs or was achieved by artificial insemination, there are also postzygotic barriers that prevent the appearance of hybrid offspring.

9.5.1 Male Genitalia

There is an obvious difference in structure of endophalli among the drones of *Apis* (cf. Chap. 3, Fig. 3.2), which can undoubtedly lead to reproductive isolation (Koeniger and Koeniger 1991; Koeniger and Koeniger 2000). Differences in body size between queens and drones and differences in drone genitalia among species also occur. When mating occurs in the air, drones have to fly fast enough to catch the flying virgin queen. Queens and drones from different species cannot mate with each other because of their body size differences. The weights of drones and queens of the nine species of honeybee are listed in Table 9.2. There are some crucial species-specific factors that determine the failure of interspecific mating as shown in Table 9.2.

According to the data from available reports, as listed in Table 9.2, *A. mellifera* drones produce the greatest number of spermatozoa. It is somewhat strange that the drone of *A. mellifera* is heavier than that of the giant honeybee. Also, the spermatozoa of *A. mellifera* are longer than those of *A. dorsata*. The queens of *A. dorsata* and *A. nigrocincta* have higher mating frequencies than the queens of other *Apis* species. Besides, there are some other species-specific organs that can prevent the interspecific mating between species. For example, the drones of dwarf honeybees, *A. florea*

Table 9.3 Mating time separation of sympatric honeybee species. (Koeniger and Koeniger 2000)

Locality	Sri Lanka	Thailand	Sabah, Borneo
Reference Species	Koeniger and Wijayagunsekera 1976	Rinderer et al. 1993	Koeniger et al. 1996d
<i>A. andreniformis</i>	—	12.15–13.45	12.00–13.45
<i>A. florea</i>	12.00–14.30	14.00–16.45	—
<i>A. cerana</i>	16.15–17.15	15.15–17.30	14.00–16.15
<i>A. koschevnikovi</i>	—	—	16.45–18.30
<i>A. dorsata</i>	18.00–18.45	18.15–18.45	18.15–19.05

and *A. andreniformis*, have a basitarsus on their hind legs which serve to clasp the hind legs of the queen during mating. *A. koschevnikovi* drones have a specific sex characteristic of a hairy fringe on the margin of the tibia of the hind leg which also strengthens their connection with the queen during their copulation (Rinderer et al. 1989).

9.5.2 Drone Congregation Area Differences

Without exception, all honeybee species mate on the wing. Drones from many colonies gather in a DCA to form a drone cloud waiting for virgin queens. Different species and even subspecies have different DCAs. *A. mellifera* drones form their clouds at heights between 5 m and 40 m according to the weather. *A. mellifera carnica* drones form their DCAs higher than those of *A. mellifera ligustica* (Koeniger and Koeniger 2001). DCAs of *A. cerana* are usually near the top of big trees (Punchihewa et al. 1990).

9.5.3 Mating Times

Although different species of honeybees occurring in the same area tend to rear their new queens nearly at the same season given suitable weather and food resources, the species have differing mating times. The mating times of several sympatric species in some areas are listed in Table 9.3. In Sri Lanka, where several honeybee species occur, *A. florea* mates earlier than *A. cerana* and *A. dorsata*, while in Thailand, *A. andreniformis* is the earliest, and on Sabah Province of Malaysia, several species there have mating times similar to those they have in Thailand.

As is evident from Table 9.3, the same species in different locations may differ in mating times, but they do have a clear mating sequence when they occur with other species: the dwarf species, *A. andreniformis* and *A. florea*, mate early, followed by cavity-nesting and middle sized honeybees, *A. cerana* and *A. koschevnikovi*. The drones of *A. dorsata* perform mating flights at dusk at all locations (Koeniger et al. 1994b).

Different male genitalia, different DCAs, combined with different mating times strongly indicate that the Asian honeybees have solved the mating intervention problem in the process of speciation. However, the balance can be easily broken when *A. mellifera* is present, having the same flight time and the same reaction to the sex attractant at the same congregation areas.

It was shown that *A. mellifera* drones actually mate with *A. cerana* queens, though with a noxious effect on the queen. A young *A. cerana* queen was found with its damaged sting chamber firmly blocked by the mating sign of an *A. mellifera* drone (Ruttner and Maul 1983), thereby indicating that no premating barrier exists between these two species as is the case between other species (Ruttner 1988). Some researchers found that *A. mellifera* drones fly into the DCA of *A. cerana* and actually copulate with *A. cerana* queens (Yoshida 1994). In China, it has been reported that when commercial *A. mellifera* apiaries arrived, there was a significantly higher loss rate of *A. cerana* virgin queens during their mating flights. Thus it has even been suggested that these phenomena can be regarded as a not yet finished stage of speciation (Ruttner 1988). Moreover, Koeniger (1976a) also inferred that the mating intervention from *A. mellifera* may exist on *A. florea* in the tropic areas in Asia.

9.5.4 Artificial Insemination

Now that interspecific mating can actually happen under natural conditions, the question arises as to what happens after this mating and whether the hybrid offspring is produced. None of the eggs hatch because of post-zygotic barriers between the species. Artificial insemination between *A. cerana* and *A. mellifera* has been applied by researchers (Ruttner 1969; Ruttner and Maul 1983; Woyke 1973; Koeniger et al. 1996b; Koeniger and Koeniger 2000; Phiancharoen et al. 2004), but no hybrids have been obtained thus far. Ruttner (1988) described the detailed developmental process in eggs laid by the queen after heterospecific instrumental insemination. The heterospecific spermatozoa can enter the spermatheca, are able to survive there, and can fertilize eggs. Twenty-four hours after fertilization, cleavage is observed to the blastula stage of the zygote. Then, however, the cell walls start to disintegrate and nuclei migrate into the secondary periplasm to accumulate in the antero-ventral part of the zygote and then to degenerate completely later on. Thus no hybrid larva or imago ever develops.

Yoshida (1994) used the mixed semen of *A. cerana* and *A. mellifera* drones to inseminate *A. mellifera* virgin queens. By using different mixed ratios of the two specific spermatozoa (approximate spermatozoa concentration ratio of 3 mm³ of *A. mellifera* semen + 1 mm³ *A. cerana* semen (9:2), 2 mm³ of *A. mellifera* semen + 2 mm³ of *A. cerana* semen (6:4), 1 mm³ of *A. mellifera* semen + 3 mm³ of *A. cerana* semen (3:6) was 79.5, 53.6, and 26.5 %, respectively), he found that the hatchability after the queen laid eggs produced only *A. mellifera* workers, interspecific fertilization resulted in nonviable larvae. Koeniger (1996) reported that interspecific hybrids between *A. cerana* and *A. koschevnikovi* produced by artificial insemination have low fertility and the hybrid colonies are probably nonviable.

Phiancharoen et al. (2004) used spermatozoa from drones of four species (*A. mellifera*, *A. cerana*, *A. florea*, and *A. dorsata*) to respectively inseminate *A. mellifera* queens. They studied survival rate of each specific sperm type and the rate of eggs fertilized by each specific spermatozoon. The results showed that nearly 100 % of *A. cerana* and *A. mellifera* spermatozoa were still alive four weeks after insemination, but the motility of *A. florea* and *A. dorsata* spermatozoa decreased to a large extent, 83.4 and 61.2 % respectively, after 3 days and only a small proportion was still alive in the queens' spermathecae. As for fertilization rate, 57 % of *A. mellifera* eggs were fertilized by *A. mellifera* spermatozoa, 40 % eggs by *A. cerana* and *A. florea* spermatozoa, while less than 20 % by *A. dorsata* spermatozoa. The fluid in the queen's spermatheca played an important role in the survival rate and fertilization success rate of the heterospecific spermatozoa, but no interspecific hybrid offspring emerged.

9.6 The Impact of Introduction of *Apis mellifera* to Asia

With the development of a beekeeping industry, honeybees, particularly *A. mellifera*, were introduced into many areas of Asia for bee products such as honey, pollen, royal jelly, and propolis, etc. However, as the business benefits from the introduction of *A. mellifera* colonies grew, many problems emerged. As mentioned earlier, these included foraging competition, mating interference, robbing, and the transmission of diseases, etc. The introduction of *A. mellifera* colonies has also had an enormous impact on the native honeybee species in some areas of Asia (Japan: Sakagami 1959a; India: Atwal and Sharma 1971; China: Ji et al. 2003; Yu and Han 2003; Yang 2005; Nepal: Partap 1998).

A. mellifera was first introduced in China in the 1920s (Kuang and Kuang 2002). On introduction, this western honeybee proved adaptable to a new environment and produced higher yields of bee products and also royal jelly and propolis which cannot be collected from *A. cerana* colonies because of their extremely low productivity. Since then, this productive species of honeybee began to be widely adopted in Chinese beekeeping.

While enjoying the high profits of these bees, the negative aspects have been widely neglected and few if any had realized the strong impact of *A. mellifera* on the environment and the local honeybees, especially *A. cerana*, until the 1980s. An investigation was launched and conducted by the *A. cerana* Association of China. The results showed that *A. cerana* has become extinct in the Daxinganling forest areas in the northeast and in Xin Jiang province in the northwest. In the Northeast Plain and North-China Plain areas, all of the *A. cerana* bees in man-made hives have absconded (Yang et al. 1982). In the whole northeast zone, *A. cerana* bees can be found only in the Changbai mountain areas in wild and man-made hives. The plain of drainage area of the Yangtze River where millions of *A. cerana* colonies were kept in the past are now hard to find. In the southern provinces such as Jiangxi, Hunan, Fujian, Guangdong, Guangxi and Hainan, there are still many *A. cerana* colonies but their distribution area has shrunk greatly. Compared with these areas, *A. cerana*

colonies in the southwest are in a better condition, particularly in mountainous areas where many *A. cerana* bees can be found living in tree holes, caves, and man-made hives in Yunnan province and Tibet (Yang et al. 1982).

In conclusion, the introduction of *A. mellifera* caused great losses of *A. cerana* colonies. The population of *A. cerana* colonies is now estimated at not more than one million, a decrease of some 60 % compared with the number before the introduction of *A. mellifera*, and their distribution has shrunk by 75 % (Yang 2005).

In the case of the introduction of *A. mellifera* in Asia, as early as 1959, Sakagami had noticed the impact of *A. mellifera* on *A. cerana* in Japan. In Nepal, Partap (1998) reported that plants and fruits were in shortage of pollination because of the population decrease of *A. cerana* bees, which was caused by the introduction of *A. mellifera*. Also, in Europe, with the rapid development of beekeeping at the beginning of the twentieth century, many beekeepers preferred to raise some subspecies such as *A. mellifera ligustica* and introduced them from other areas, which caused the local extinction of native subspecies (Ruttner 1988).

Moritz et al. (2005) recognized the severe disaster caused by the introduction of *A. mellifera* to tropical ecological systems and pointed out that local honeybees or other pollinators suffered from the introduced species through food competition or diseases. This resulted in a reduction of biodiversity and an imbalance of the whole ecological system.

During the mating season, both the virgin queens of *A. cerana* and *A. mellifera* can attract heterospecific drones (Yang 2001; Ji et al. 2003; Wang et al. 2003). However, the *A. mellifera* drones, which are much stronger fliers than *A. cerana* drones, can trap the *A. cerana* queens, although they cannot always mate with them successfully because of the differences in copulatory organs (see Chap. 3; Fig. 3.2). Their encirclement behavior can inhibit successful mating between *A. cerana* queens and drones. In some areas with very many *A. mellifera* colonies, most of the virgin *A. cerana* queens were trapped by *A. mellifera* drones, and only 16 % of *A. cerana* queens were able to mate successfully. More than 80 % *A. mellifera* queens could successfully mate with conspecific drones although there was interference by *A. cerana* drones (Wang et al. 2003). This resulted in the population decline of *A. cerana* bees in some areas in recent years. In some areas they are threatened because their declining population is insufficient to support the community and honeybees are dying out. The decrease or extinction of the native honeybees is a definite threat to the balance of ecology and some plant species could also become extinct because of insufficient pollination (Yang 2005).

9.7 Mixed-Species Colonies

The cavity-nesting honeybee species share several common morphological and behavioral characters and can be kept in the same colonies with heterospecific queens. Thus far, three types of mixed-species colonies: *A. cerana* with *A. koschevnikovi*, *A. cerana* with *A. nuluensis*, and *A. cerana* with *A. mellifera* have been successfully organized experimentally. Recently, in Thailand, a super-mixed colony of *A. florea*,

A. mellifera, *A. cerana* and *A. dorsata* was set up, but only lasted several weeks and then absconded all together. No biological research has been done with this kind of super mixed colony.

9.7.1 Mixed Colonies of *Apis cerana* and *Apis koschevnikovi*

Mixed colonies of *A. cerana* workers with an *A. koschevnikovi* queen were organized by Koeniger et al. (1996c). They grafted young larvae of *A. cerana* and *A. koschevnikovi* simultaneously into artificial queen cells and inserted them into queenless colonies of either *A. cerana* or *A. koschevnikovi*. Not unexpectedly, all colonies preferred to rear conspecific larvae, but *A. cerana* colonies seemed more selective than *A. koschevnikovi* colonies against heterospecific larvae. Only 4 out of 102 (4 %) *A. koschevnikovi* queens successfully emerged from *A. cerana* colonies, while 30 out of 140 (21 %) *A. cerana* larvae developed into adult queens in an *A. koschevnikovi* colony.

To set up mixed colonies, nearly emerging virgin queens in queen cells of either of the two species were introduced into heterospecific queenless colonies. In *A. koschevnikovi* colonies, all of the *A. cerana* queen cells were destroyed and the queens were killed; while a few (4 out of 18) *A. koschevnikovi* queens were accepted by the *A. cerana* colonies and three of them succeeded in mating and laying eggs. Although these queens were in heterospecific colonies, they mated with their own specific drones. Interestingly, the drones of *A. koschevnikovi* can also find their own species-specific mating times even when they were reared in *A. cerana* colonies (Koeniger et al. 1994a). The mated *A. koschevnikovi* queens laid eggs and the emerged bees were successfully reared by *A. cerana* worker bees, thus the *A. cerana* host colonies were gradually transformed into *A. koschevnikovi* colonies.

9.7.2 Mixed Colonies of *Apis cerana* and *Apis nuluensis*

De Guzman et al. (1996) set up a mixed colony of *A. cerana* and *A. nuluensis* containing brood combs and adult bees from one colony of *A. nuluensis* from one of the high mountains of Sabah, Malaysia in Borneo into a queenless colony of *A. cerana* 200 km away. It was unusual that the adult workers did not attack each other. The authors investigated only the *Varroa* mites in this mixed colony and *Varroa jacobsoni* Oudemans and *Varroa underwoodi* were found. There have been no further reports about this kind of mixed colony.

9.7.3 Mixed Colonies of *Apis cerana* and *Apis mellifera*

A. cerana and *A. mellifera* are very closely related and very similar both in morphology and behavior (Table 9.4–9.5) to the extent that there was once doubt if they

Table 9.4 Summary of differences in body size and nesting behavior among Asian honeybee and for comparison, *Apis mellifera ligustica*

Species	Body mass (mg)	Thoracic mass (mg)	Colony traits			Reference
			Number of workers	Number of combs	Nest site	
<i>A. florea</i>	22.6	7.6	6,000	1	Exposed	Seeley et al. 1982
<i>A. cerana</i>	43.6	15.3	8,000	5–6	Cavity	
<i>A. dorsata</i>	118.0	45.5	> 35,000	1	Exposed	
<i>A. mellifera</i>	77.2	29.1	15,000	5–8	Cavity	
						Seeley 1986; Seeley and Morse 1976

Table 9.5 Flight speeds and wing-loading of honey-bees

Species	Flight speed (Ms ⁻¹)	Wing-loading (Nm ⁻¹)	Reference
<i>A. florea</i>	4–81	7–28	Dyer and Seeley 1987
<i>A. cerana</i>	7–17	7–84	
<i>A. dorsata</i>	8–12	13–02	
<i>A. mellifera</i>	12–61	14–21	Seeley 1986

were distinct species (Ruttner and Maul 1983). Researchers and beekeepers have long wanted to hybridize them. For example, Atwal and Sharma (1968) introduced *A. mellifera* queens into *A. cerana* colonies and found that the introduction was successful if the *A. cerana* workers were no more than a week old. The introduced queen could lay eggs in the host colonies and the eggs hatched into larvae. *A. cerana* workers attended to them and they pupated and emerged as adults. Once the *A. mellifera* workers assumed field duties, they worked in harmony with the host *A. cerana* workers.

Studies show that young worker bees may lack pheromones and can be accepted by other colonies (Pham-Delegue et al. 1993; Laloï et al. 2001). So it is possible to exchange brood combs between colonies. Firstly, previously prepared empty combs are added to strong *A. mellifera* colonies which are then checked every day until the combs are filled with eggs so the emergence date for adults can be calculated. These brood combs are kept in nurse colonies until the adult bees are just about to emerge and then removed and introduced into queenright *A. cerana* colonies. When they emerge, the numbers of adult workers of the two species are about even, and no fighting was seen on opening the mixed-species colonies nor were dead *A. mellifera* workers found at the entrances.

9.8 Queen Rearing

Tan et al. (2006) studied queen rearing in mixed colonies to assess the effect of food on the development of offspring. *A. cerana* larvae were grafted for queen rearing into two of these mixed-species colonies. Similarly, *A. cerana* larvae and

A. mellifera larvae were also grafted conspecifically as controls. The success rate of *A. cerana* queen rearing in the test colonies was 64.5 %, surpassing all previous attempts at interspecific queen rearing, in which single-species host colonies were used (Oschmann 1965; Dhaliwal and Atwal 1970; Oku and Ono 1990; Potichot et al. 1993). After emergence, all virgin queens obtained from the three groups ($N = 90$) were measured morphometrically. The *A. cerana* queens from the mixed-species colonies differed significantly in size and pigmentation from the *A. cerana* control queens and closely approximated the *A. mellifera* queens. It is inferred that these changes in the *A. cerana* queens reared in the mixed-species colonies can be attributed to feeding by heterospecific nurse bees and/or chemical differences in royal jelly, the data showed a strong impact of environment on the development of queens. The results further suggest that in honeybees the cues for brood recognition can be learned by heterospecific workers after eclosion.

9.9 Retinue Behavior

The retinue behavior of worker bees of *A. cerana cerana* and *A. mellifera ligustica* in two types of mixed-species colonies was studied (Yang et al. 2009). In mixed colonies headed by an *A. cerana* queen almost equal numbers of *A. cerana* and *A. mellifera* workers attended to the *A. cerana* queen; while in mixed colonies headed by an *A. mellifera* queen significantly fewer *A. cerana* workers were attracted than *A. mellifera* workers. The pheromones 9-ODA, 9-HDA and 10-HDA of the queens were significantly different and the workers did not show avoidance behavior to either heterospecific queens. Both species of workers were attracted by the queens and engaged in retinue behavior, suggesting that the retinue response was not related to a specific queen pheromone or colony environment. This non-specific queen retinue behavior in the mixed colonies indicates that the queen pheromones can be transmitted among the workers from the two species without any obstacles. We conclude that retinue behavior itself, as well as the pheromones of the queens, that induce this behavior are both primitive, conserved traits that preceded speciation in apine bees.

9.10 Ovary Activation

The workers in mixed colonies show different degrees of ovarian activation. *A. cerana* workers showed significantly greater ovarian activation in queenright mixed-species colonies than in conspecific queenright colonies. There was significantly greater ovary activation in *A. cerana* workers in mixed-species colonies headed by *A. mellifera* queens than *A. mellifera* workers in mixed-species colonies headed by *A. cerana* queens. *A. mellifera* workers in conspecific queenless colonies showed significantly greater ovarian activation than those in mixed-species queenless colonies. Quantification of the chemical components of mandibular gland pheromones of queens of the

two species showed that they are similar. Combined, the results show that although queen signals have been preserved between the two species, the threshold of queen pheromone necessary to suppress ovary activation in *A. cerana* is higher than that for temperate *A. mellifera* (Tan et al. 2009).

9.11 Interspecific Communication

Among the most interesting of the interspecific interactions between *A. cerana* and *A. mellifera* workers in the same colony is the mechanism of interspecific communication. Honeybees have a dance language by which information about food resources can be transferred from successful foragers to nestmates (von Frisch 1967; Dyer 2002).

The question arises: can the dance followers of one species understand the dances performed by the foragers of the other species although the structure of the dance language is very similar among species of honeybees? (Lindauer 1956). Studies have shown that the dance language not only differs among species in the genus of *Apis*, but different races of the same species may also have dialects (Steche 1957; Sarma et al. 2004). For example, Lindauer (1956) observed the Asian species *A. cerana*, *A. florea*, and *A. dorsata* and reported that there were differences in the distance at which dancers changed from round dances to waggle dances. The transition distance was much closer for the Asian species, e.g., he reported that *A. florea* started wagging when the feeder was only 5 m from the hive. Lindauer (1956) and Boch (1957) also reported interspecific/interracial differences in the dance tempo (dance circuits per 15 s) at a given distance. For the same distance, different races or species would execute a different number of circuits per unit time.

Thus, the concept of dialects in the honeybee dance language was established which basically pointed to two differences in the dances by different species and races, firstly in the flight distance at which the dancers start performing waggle dances instead of round dances, and secondly in the circuit duration of the waggle dance performed by dancers for a given flight distance.

So we understand that although the structure of the dance language is very similar among species of honeybees, communication of the distance component of the message varies both intraspecifically and interspecifically. However, it is not known whether different honeybee species would attend interspecific waggle dances and, if so, whether they can decipher such dances. So far, two reports have tried to answer this question, and both have found that *A. cerana* foragers could decode the dances of *A. mellifera* to successfully locate an indicated food source, by using mixed-species colonies of *A. cerana* and *A. mellifera* (Su et al. 2008; Tan et al. 2008). More recently, Tan et al. (2008) found that *A. mellifera* foragers can also be recruited to the experimental feeder by *A. cerana* dancers.

9.12 Comb Building Cooperation

Cooperation in comb building in mixed colonies has also been investigated (Yang et al. 2010a). Two types of cell sized (*A. cerana* and *A. mellifera*) foundation made from wax of these two species were inserted into mixed colonies to study cooperation in comb construction. The mixed colonies did not discriminate between the wax types, but the *A. cerana* cell-size foundation was modified during comb building by the cooperative efforts of the workers of both species. In pure *A. cerana* colonies workers did not accept any foundation, but were stimulated by *A. mellifera* workers to secrete wax and build on the foundation in mixed colonies. The task of comb building is actually performed by small groups of workers of the two species. In this way, the two species cooperate in comb building and can construct nearly normal combs, even though they contain many cells of irregular shapes (Yang et al. 2010a).

9.13 Thermoregulation

A. cerana and *A. mellifera* normally display different strategies in cooling their nests, raising the question whether they would coordinate their efforts to achieve stable thermoregulation in mixed colonies. The results show that the normal temperatures in the brood area in mixed colonies are more similar to those of pure *A. cerana* colonies than pure *A. mellifera* colonies. Under heat stress, *A. cerana* workers are more sensitive, and initiate fanning earlier than *A. mellifera* workers. In mixed colonies, the former become the main force for thermoregulation. When worker bees of both species were fanning together at the entrance, their own species-specific postures were adopted, but due to a significantly smaller number of *A. mellifera* workers engaged in fanning, the cooling efficiency of mixed colonies were closest to that of pure *A. cerana* colonies (Yang et al. 2010b).

9.14 Defensive Behavior of Honeybees

When vespine wasps hawk honeybees at their nest entrances, alerted and poised guard bees of *A. cerana* and *A. mellifera* in the mixed colonies have average thoracic temperatures slightly above 24 °C. *A. cerana* workers assume their species-specific wing shimmering and raise their body temperature up to about 29 °C, while *A. mellifera* guard bees neither show significant body temperature increase nor wing shimmering. However, when faced with persistent hawking wasps, guard bees of both species raise their thoracic temperatures and form a ball around it, the core temperature of the mixed-species balls were about 45 °C, which is not significantly different from the heat ball made up by only pure species. *A. cerana* bees engulf the ball tighter in the inner space while *A. mellifera* bees can be seen more likely roaming at the outer space. This result shows that the defense behaviors of the two species are based on their species-specific adaptations in the evolutionary background (Tan et al. 2010).

In conclusion, mixed colonies offer us a unique probe to study interspecific relations between species of honeybees. Behaviors in the mixed colonies confirm that these two species *A. cerana* and *A. mellifera* are indeed very closely related species. Also, it provides us more information about these two societies. It may also prove useful in finding a way to solve the problem following the introduction of western bees in Asia.

I (Abrol 2006) studied the defensive behavior of *A. cerana* F. against predatory wasps *Vespa velutina* and *Vespa magnifica*. The honeybee *A. cerana* showed a well organized defense and killed more number of predatory wasps by exhibiting well organized balling behavior as compared to *A. mellifera* L. The bee mortality was higher when fewer wasps visited the apiary due to an unorganized defense. However, when the intensity of attack was severe, fewer bees and more wasps were killed due to an organized defense. The defensive behavior of *A. mellifera*, on the other hand, was not well organized and wasps inflicted heavy losses on their colonies. During hornet attacks on *A. mellifera* colonies, up to 150 workers form a close cluster at and around the hive entrance. When a hornet approaches the cluster, one or two bees may leave the cluster and walk rather aggressively towards the hornet, but this behavior does not repel the hornet, which seizes one of the bees and flies off with it. Guard bees seldom attack the hornets and appear incapable of seizing and holding a captured hornet firmly enough for it to be killed. The bees, in an attempt to capture the wasp, chased it in low numbers and were killed in the process. Unlike *A. cerana*, they do not attack en masse and suffered more casualties. Wasps were also found to adopt counter attack strategies to maximize gains and minimize losses in the following ways:

- (a) They preferred *A. mellifera* bees as they met very little resistance due to less-organized defense. In the case of weak colonies, they could enter the hives and eat the bees, brood, nectar stores etc.—whatever was in their way.
- (b) Some of the wasps continued flying around the back of the hive in order to prey on individual bees, without any apparent danger from the guard bees. By this method, they were able to prey on a large number of bees with minimum risk.
- (c) They captured the bees whilst in flight and/or foraging on flowers.
- (d) Some hornets in groups of 3–4 remained hovering near the hive entrance to distract the attention of the bees; when the bees chased a lone wasp, the other wasps could ambush the bees.
- (e) Some hornets remained hovering in one spot and pounced on incoming bees laden with nectar or pollen. On the bee side, their defensive strategy is highly cost-effective in terms of energy involved, loss of time, and collection of stores. In a ball, an average of 175.67 ± 37.35 bees are involved and the balling continues for an average of 58.52 ± 10.49 min which results in a loss of more than 165 bee hours/ball/day. Additionally, if on an average, 2–3 balls are formed per day per colony, the losses could be much higher. Evidently, in addition to the defensive strategy of the bees, some control measures such as using traps, baiting of wasps, physical killing by flapping, killing of queens, and destruction of wasp nests could help the colony to collect more nectar and pollen resources.

Tan et al. (2012) reported that when a prey animal displays to a predator, the prey benefits because it is less likely to be attacked, and the predator benefits because it can break off an attack that is unlikely to succeed because the prey has been alerted. They argue that an “I see you” signal has coevolved between the Asian hive bee, *A. cerana*, and its hornet predator, *Vespa velutina*. When a hornet approaches a bee colony, guards perform a shaking movement that repels the hornet. To test whether this is an “I see you” display, they exposed colonies to free-flying and tethered hornets and tethered butterflies. The intensity of the shaking was correlated with the hornet’s proximity, whereas guard bees barely responded to a nonthreatening butterfly. The signal is likely to be honest, because the bees can kill the hornet by collective mobbing if it lands on the entrance. The Western honeybee, *A. mellifera*, which has not evolved in the presence of Asian hornets, does not produce the signal and is ineffective at killing hornets by collective mobbing. We also found that hornets were more successful at catching *A. mellifera* than *A. cerana* bees at the hive entrance.

9.15 Comb Wax and its Mechanical Properties

Buchwald et al. (2006) studied the mechanical properties of waxes from four honeybee species: *Apis mellifera* L., *Apis andreniformis* L., *Apis dorsata* L. and two subspecies of *A. cerana* L. *A. dorsata* wax was stiffer and had a higher yield stress and stress at the proportional limit than all of the other waxes. The waxes of *A. cerana* and *A. mellifera* had intermediate strength and stiffness, and *A. andreniformis* wax was the least strong, stiff, and resilient. All of the waxes had similar strain values at the proportional limit and yield point. The observed differences in wax mechanical properties correlate with the nesting ecology of these species. *A. mellifera* and *A. cerana* nest in cavities that protect the nest from environmental stresses, whereas the species with the strongest and stiffest wax, *A. dorsata*, constructs relatively heavy nests attached to branches of tall trees, exposing them to substantially greater mechanical forces. The wax of *A. andreniformis* was the least strong, stiff, and resilient, and their nests have low masses relative to other species in the genus and, although not built in cavities, are constructed on lower, often shielded branches that can absorb the forces of wind and rain.

9.16 Foraging

9.16.1 Competition for Floral Resources

If the two species compete for a resource, it must be limiting in such a way that an increase in resource harvest by one species correspond to the diminished harvest by the other. Competition also occurs if resource harvest remains stable but harvest cost increases. The evidence for interspecific competition for food is usually circumstantial, and, despite extensive treatment of the subject, few field studies

clearly demonstrate its importance. The old world honeybee *A. mellifera* is thought to displace native pollinators from both floral resources and geographic areas. The importance of interspecific competition for the structure and diversity of communities has attracted much attention and controversy during recent decades (Connell 1983; Schoener 1983; Abrams et al. 1986; Trepl 1994). Within a particular habitat, different species of bee often use the same resource (pollen, nectar, floral oils, and so on). Should the resource become insufficiently abundant to fulfill the ecological requirements of all species involved, it becomes limiting, and the distribution and abundance of one or more of the species may be reduced or excluded through competition. The greater the habitat overlap among species in their ecological requirements, the more intensively they are expected to compete (Roubik 1989, 1996; Thorp 1996). Interspecific competition for limited resources affects the reproductive success and survival of the species involved and may explain evolutionary specialization and niche differentiation. Although it is difficult to prove the evolutionary significance of interspecific competition observed in the field (Schoener 1986), both experiments in simplified and artificial habitats (e.g., Gause 1969), and mathematical models (e.g., Scudo and Volterra 1978; Chesson 1994) have shown its possible influence. Nectar and pollen feeders, especially bees (Hymenoptera: Apoidea), are often assumed to be strongly affected by interspecific competition for the high-quality food resources provided by flowering plants to attract pollinators (e.g., Eickwort and Ginsberg 1980; Schaffer et al. 1979, 1983; Plowright and Laverty 1984; Schoener 1986; Westrich 1989; Corbet et al. 1995; Sudgen et al. 1996).

Researchers have recently obtained evidence of competition between bee species in natural situations. Inouye (1978) studied two species of bumblebees that were foraging primarily on different flower species in Colorado. When he removed one bee species, one flower species was left unused. Foraging patterns of the other bee species shifted to include more of the abandoned flower species. Morse (1977) noted that workers of two bumblebee species were partitioned along sprays of golden-rod, *Solidago canadensis*. Apparently, *Bombus ternarius* workers avoided the larger *B. terricola* workers (Morse 1977). Morse (1978) recorded interspecific displacement of bumblebee workers on roses as well. From his data on nectar depletion and resource partitioning in Maine, Heinrich (1972) inferred that four bumblebee species competed for nectar. Johnson and Hubbell (1974, 1975) reported aggressive defense of favorable resource sites by certain stingless bees. The spacing of nests of these bees may reflect competition for floral resources (Hubbell and Johnson 1977).

A. cerana is sympatric in distribution and can coexist with the two other species of Asiatic honeybees, *A. dorsata* and *A. florea*, without any adverse ecological consequences. Chahal et al. (1986) found that *A. florea* showed aggressiveness towards *A. mellifera* both at hive and on flowers. Partap (2000) studied the foraging competition between *A. cerana* and *A. mellifera* on four crops blooming simultaneously and found that *A. cerana* foragers were less abundant when *A. mellifera* bees were in the plots in all the crops. Removal of *A. mellifera* colony from the plots resulted in reversal of the abundance trend among both the species. After removal of *A. mellifera*, the number of *A. cerana* foragers increase significantly from 12.6 to 20.8 in mustard, 12.3 to 18.3 in broad leaf mustard, 18.4 to 28.3 in cauliflower and from 9.7

to 16.2 in raddish. Evidently, presence of *A. mellifera* in the field reduces the number of *A. cerana* bees in the field. The two species try to displace each other rather than visiting/pollinating flowers. It is, therefore, inferred that the presence of both species adversely affect the pollinating efficiency of both the bees. He further found that removal of *A. mellifera* resulted in significant increase in time spent per flower, number of flowers visited per minute, weight of pollen load, number of pollen collectors, but after its removal number of pollen loads from other crops decreased significantly. Numerous interactions among the species were noted, small individuals generally attacking larger ones. *A. dorsata*, however, was attacked only by *A. cerana*, never by the other two species. In case of *A. florea*, their small size does not deter them from showing aggression towards other *Apis* species at a food source. When foraging at dishes containing sugar syrup, *A. florea* was as successful (or more so) than *A. cerana* and *A. dorsata* in maintaining its station there and inducing the departure of bees of other species. However *A. florea* and *A. cerana* workers sometimes forage together at an artificial food source without showing aggression.

9.16.2 Foraging Periodicity of Bees

Bees of the genus *Apis* are important foragers of nectar and pollen resources. The European honeybee, *A. mellifera*, has been well studied with respect to its sensory abilities, learning behavior, and role as pollinators. Of the four species, the giant honeybee *A. dorsata* (which forages during moonlit nights) has the lowest spatial resolution and the most sensitive eyes, followed by *A. mellifera*, *A. cerana* and the dwarf honeybee, *A. florea* (which has the smallest acceptance angles and the least sensitive eyes). Moreover, unlike the strictly diurnal *A. cerana* and *A. florea*, *A. dorsata* possess large ocelli, a feature that it shares with all dim-light bees (Table 9.6). However, the eyes of the facultatively nocturnal *A. dorsata* are much less sensitive than those of known obligately nocturnal bees such as *Megalopta genalis* in Panama and *Xylocopa tranquebarica* in India. The differences in sensitivity between the eyes of *A. dorsata* and other strictly diurnal *Apis* species cannot alone explain why the former is able to fly, orient, and forage at half-moon light levels. We assume that additional neuronal adaptations, as has been proposed for *A. mellifera*, *M. genalis*, and *X. tranquebarica*, might exist in *A. dorsata*.

9.16.3 Foraging Preferences

There is relatively little written about the floral preferences of *A. cerana*. In 1958, Miyamoto noted that *A. cerana* in Japan visited a wider variety of plant species, including natives, compared to the limited floral preferences of *A. mellifera* (Ruttner 1988). In a 1968 German study of honey from *A. cerana* and *A. mellifera*, clear differences in the pollen spectrum were collected by the two species despite them

Table 9.6 Ecological data, body, ocellus and eye measurements, and the acceptance angles and optical sensitivities of single ommatidia in four species of honeybees of genus *Apis*

	<i>A. mellifera</i>	<i>A. cerana</i>	<i>A. florea</i>	<i>A. dorsata</i>
Ecological data				
Temperature range (°C)	> 11 ^g	> 10.5 ^a	18–43 ^a	> 15 ^a
Light intensity	Half-moon in some subspecies ^g	Daylight	Daylight	Half-moon ^h
Body and eye measurements				
Intertegular width (mm)	3.2	3.0 ± 0.05	2.2 ± 0.07	3.9 ± 0.2
Tongue length (mm)	5.8–7.1 ^c	4.53 ± 0.01 ^b	3.41 ± 0.02 ^b	6.14 ± 0.04 ^b
Ocellus diameter (mm)	0.27 ^d	0.26	0.24	0.38 ± 0.02
Eye length (mm)	2.6 ^d	2.1 ± 0.1	1.7 ± 0.05	2.8 ± 0.1
Length of sensory hair on the eyes (μm)	275 ⁱ	125 ± 14	50 ± 6	100 ± 7
Facet number per eye	5432 ^f 4752 ^e	5004	3484	6394
Max. ommatidial diameter <i>D</i> (μm)	20 ^e	24.5	19.5	29.5
Corneal thickness (μm)	27–38 ^d	27–35	25	46
Crystalline cone length (μm)	55	50	36	55
Focal length <i>f</i> (μm)	66 ^e	84	79	104
Distal rhabdom diameter <i>d</i> (μm)	2.0 ^e	1.8	1.5	3.2
Acceptance angle Δρ (degrees)	1.7 ^e	1.2	1.1	1.8
Rhabdom length <i>l</i> (μm)	320 ^e	270	190	260
Optical sensitivity to white light <i>S</i> (μm ² sr)	0.11 ^e	0.07	0.03	0.21

Data that were not obtained in this study are from ^aCorlett 2004; ^bOldroyd et al. 1992; ^cBöttcher 1977; ^dRibi et al. 1989; ^eGreiner et al. 2004; ^fSeidl and Kaiser 1981; ^gFletcher 1978 on *A. m. adansonii*, ^hDyer 1985; ⁱNeese 1965

operating at the same time and in the same vicinity (Ruttner 1988). Some studies claim that *A. mellifera* is clearly dominant over *A. cerana* in common feeding locations (Sakagami 1959a; Dhaliwal and Atwal 1970; Ruttner 1988). Another, more recent study suggests the opposite. In the Solomon Islands, substantial losses of exotic *A. mellifera* honeybees were attributed to *A. cerana* robbing *A. mellifera* hives and increasing competition for floral resources (Anderson et al. 1994).

9.16.4 Foraging in Disturbed Habitats

More widely accepted is that *A. cerana* does well in disturbed or extensively modified habitats. For example, in Hong Kong, *A. cerana* visits 86 % of plant species and pollinates so successfully as to maintain that island's diverse flora (Corlett 2001; Oldroyd and Wongsiri 2006). In one study of *A. mellifera* conducted in the Philippines, researchers found that the endemic bees, *A. cerana* and *A. dorsata*, negatively

affected the growth of *A. mellifera* colonies in a forest ecosystem by aggression and robbing of stores. However, this finding was not duplicated for colonies studied in industrial or agricultural areas. Rather, the population growth of *A. mellifera* in an agro-ecosystem was significantly higher than in the industrial or forest environments. The abundance of melliferous plants in the agro-ecosystem enhanced the population build-up of *A. mellifera*. They conclude that in spite of the diversity in a forest ecosystem, the exotic species *A. mellifera* failed to exploit the nectar and pollen sources of most plant species. This indicates that *A. mellifera* did not adapt to natural forest conditions in the tropics (Manila-Fajardo and Cervancia 2003).

9.16.5 Resource Partitioning

Sharma et al. (2000) studied resource partitioning in *A. mellifera* and *A. cerana* and found that of the 23 plant species studied, *A. mellifera* and *A. cerana* avoided competition by visiting the plants in differing intensities. During spring, *A. mellifera* preferred *Rubus ellipticus* (7.85 bees) and *Prunus armeniaca* (7.6 bees) whereas *A. cerana* was more active on *Malus domestica* (9.91 bees). Partap et al. (2000) found that *A. cerana* workers started foraging earlier in the morning (07.31 on peach and 08.12 h on plum) compared to *A. mellifera* worker bees that started foraging at 08.01 h at peach and 08.37 h at plum. In the evening, *A. mellifera* stopped earlier (17.35 h on peach and 17.02 h on plum compared to *A. cerana* (18.06 h on peach and 17.51 h on plum). Sihag (2000a) reported that *A. florea* starts foraging when the ambient temperature surpasses 18 °C and continues foraging until ambient temperature approaches 43 °C. Maximum foraging activity is shown at 30–40 °C. These ranges are higher than those shown by *A. mellifera* and *A. dorsata*. In case of *A. dorsata* (Sihag 2000b), foraging commences when temperature surpasses 16 °C and continues foraging to around 40 °C. Maximum foraging activity is shown at 25–40 °C. These ranges are lower than *A. florea* but higher than *A. mellifera*.

9.16.6 Influence of Honeybees on Other Bee Species

Pearson (1933) suggested that the honeybee, an introduced species in the New World, influences native bees in a profound manner. Several authors have since presented experimental evidence of competition between honeybees and wild bees for flower resources. Roubik (1978) introduced hives of Africanized honeybees near patches of various flower species in French Guiana. The honeybees appeared to displace stingless bees from some of the flower species. They also nonaggressively displaced stingless bees from artificial feeders, even though some of the displaced meliponines were aggressive species (Roubik 1980). Holmes (1961) exposed frames of honey to foraging honey and bumblebees. Bumblebees retreated from direct encounters with

honeybees and worked the opposite side of the frame from the honeybees. Displacement and even aggression between honeybees at artificial nectar sources has been reported. Kalmus (1941) trained one colony of honeybees to an artificial source of sugar. When he stopped supplying the solution (so that resources became scarce), these bees aggressively defended the food source against another colony of bees (a different strain). This sort of aggression may be common among bees in greenhouses (Lecomte 1955). *A. mellifera* aggressively displaced *A. cerana* from artificial sugar feeders in Japan (Sakagami 1959a). Interactions suggesting competition between honeybees and wild bees in more natural situations have also been reported. Wratt (1968) found that the number of *Bombus ruderatus* foragers carrying pollen on plots of red clover in New Zealand decreased with increasing temperature, apparently due to competition by *Apis*. Benest (1976) studied foraging by honeybees and three species of bumblebees on a field of dwarf dahlias in France. Individuals of the same species foraged together on single flowers, but bees avoided foraging on the same flower with individuals of another species. Ginsberg (1979) found that the distribution of honeybees on patches of attractive spring flowers differed from that of native bees. Honeybees apparently outcompeted native species at large flower dusters. Schaffer et al. (1979) censused *A. mellifera*, *Bombus sonorus*, and *Xylocopa arizonensis* on several stands of *Agave schottii* in Arizona. The biomass of *Apis* was greatest at the most productive stands, *Bombus* at intermediate stands, and *Xylocopa* at the least productive stands. These authors proposed a model in which honeybees crop resources at productive sites down below the threshold level at which foraging is energetically profitable for individual bumblebees or carpenter bees. In other cases, standing crops of resources may remain profitable at large productive stands, but small flower clusters may have higher nectar or pollen rewards per flower due to exploitation of large stands by *Apis*. Taken together, these studies provide strong evidence that honeybees do influence the foraging patterns of native bees by competition at resource sites.

The following impact of *A. mellifera* on *A. cerana* in the Chinese Himalayan region has been recognized:

- Competition for forage sources
- Species competition: When forage source is limited, *A. mellifera* usually invade to hives of *A. cerana* to get honey, which leads to fighting between two species. Usually the whole *A. cerana* colony will be killed by *A. mellifera*.
- Ectohormone of *A. mellifera* may affect the mating of *A. cerana* when both are in the region within 20 km. Usually the queen of *A. cerana* cannot mate due to ectohormone of *A. mellifera*, resulting in unfertilized eggs and only male bees being developed, leading to death of the whole colony.
- *A. mellifera* spreading diseases such as paralysis, American foulbrood, European foulbrood, sacbrood, and chalkbrood. Before introduction of *A. mellifera*, *A. cerana* had not been found infected with these diseases. Once infected, the spread is very fast and loss is severe. For example, in 1972, *A. cerana* was infected with sacbrood in Guangdong Province and this was spread very quickly throughout China, resulted in death of many colonies of *A. cerana*. In Yunnan

Table 9.7 Summary of *Apis* species in Chinese Himalayan region

Species/subspecies	Comb frames	Honey yield (kg)	Pollen yield (kg)	Wax yield (kg)
<i>Apis cerana cerana</i>	8–10	30	1–2	1
<i>Apis cerana indica</i>	3–5	10–15	1	0.5
<i>Apis cerana skorikovi</i>	5–8	20	1.5	0.8
<i>Apis mellifera</i>	16–20	50	3–3.5	
<i>Apis dorsata</i>	1	5–8		1
<i>Apis laboriosa</i>	1	15–20		1.5
<i>Apis florea</i>	1	1–1.5		
<i>Apis andreniformis</i>	1	1–1.5		
<i>Trigona</i> spp.		0.5		

Province only, 620,000 of the 770,000 colonies of *A. cerana* died due to this disease. From 1992, chalkbrood of *A. mellifera* is also infecting *A. cerana* and this will lead to big loss. Due to diseases, many colonies of *A. cerana* died. In this connection, some disease-persistent strains were bred and the colonies of *A. cerana* in Yunnan Province have risen to 1 million recently. The traditional bee-keeping method needs to be improved. In Yunnan, about 90 % *A. cerana* colonies are kept in the traditional hives of logs or in the wall and collection of honey was done through destroying frames, and the annual honey yield was only 1–2 kg.

9.16.7 Selection and Multiplication of *Apis cerana* Colonies

Due to the invasion of *A. mellifera* and the spread of pests and diseases, the number of *A. cerana* colonies decreased to a very low level. It was realized that if proper measures are not taken, *A. cerana* may get extinct in some time. It is also realized that if the rearing techniques can be improved to yield sufficient honey and other produces, more farmers will keep this species to increase their cash income. In this connection, selection and multiplication of disease-persistent strains have been carried out in different parts of China and very promising achievements have been achieved. For example, some good strains have been bred in Yunnan and northeastern China (Table 9.7). Some of these have been released to farmers.

From 1985, the Eastern Bee Research Institute started a program to expand improved beekeeping methods to farmers by organizing training courses. To date, the improved method has been used in over 150,000 colonies of *A. cerana*. The average honey yield is enhanced to 10–15 kg, and highest up to 72 kg. A total of 12,000 people in 3,000 farmer households have got considerable benefits from these improved beekeeping methods.

The role of pollination of insects including honeybees has not been well recognized and understood in China and need to be greatly promoted. Almost all the beekeepers or farmers keep honeybees exclusively for honey and other products though some farmers realized the role of honeybees in pollination of crops. The preliminary survey indicates that in most of the apple and pear production regions, apples and pears are

almost 100 % hand pollination, as in the case of Maoxian of Sichuan Province and one important pear production county in Shandong Province. Many farmers even thought in the past that bees eat flowers, suck “blood” of the crops, and spread diseases and pests. Local farmers usually required to be paid by the beekeepers because they claim that it is their crops that feed the bees, which is correct.

In nestmate recognition experiments, *A. cerana* was among the bee species that did not exhibit aggressive responses to the presence of other bees in their nests (Breed et al. 2007). These authors suggest that robbing of stored food may be more characteristic of *A. mellifera* than other species in the genus *Apis*. Similar reports appear in Ruttner’s (1988) account of this species. In relation to *A. mellifera*’s robbing efforts, he writes that “no effective defensive reactions are developed in *A. cerana*” (Ruttner 1988). Indeed not only can intruders pass unimpeded, but *A. cerana* bees inside a robbed colony were observed feeding the intruding *A. mellifera* robber bees.

There is divergent evidence about the size of *A. cerana* colonies. A recent report of a full sized colony described it as containing “about 1500 g bees, 700 g brood, about 4 kg of honey and about 400 g of cells filled with pollen” (Koeniger et al. 2010). Other reports cite “usual” colony sizes of 1,400–2,000 bees or between 10–20,000 bees (Makhdzir and Osman 1980 cited in Ruttner 1988:131; Okada 1985 cited in Ruttner 1988:131). Colony size seems to depend on nest cavity availability (reported further).

A. cerana tolerate a wide range of temperatures—from 5 to 45 °C; however, when compared to *A. mellifera*, at 50 °C, *A. cerana* survived for a much shorter time while at 5 °C they equaled *A. mellifera* survival rates (Verma and Edwards 1971 cited in Ruttner 1988:130). There is evidence from Ussuria, Kashmir, Japan, and China that *A. cerana* are active at lower temperatures compared to *A. mellifera* and that they are therefore more active earlier in the morning than *A. mellifera* and can start flying earlier in spring than *A. mellifera* (Ruttner 1988). However, it should be noted that these data “could be specific adaptations of certain ecotypes” (ibid p. 139) and may not be generalizable across whole species.

An important cautionary note should be observed from the outset about the behavior of *A. cerana*. Most research has been done on dead bees with fewer descriptive studies of live specimens and very few experimental studies of comparative “species characteristics”. Therefore, it is not clear whether *A. cerana* defense behavior observed in the field at one location and point in time should be generalized to other times/places or for the species as a whole (Ruttner 1988). Much has been written about colony defense behaviors and the consensus for *A. cerana* is that “it is generally reported as being mild, tolerant and timid” (Ruttner 1988) in the context of attacks from European genotypes of *A. mellifera*. However, there are some unique behaviors associated with this species associated with colony defense: (a) abdomen shaking (b) hissing (like a snake) in response to knocks or interference with the combs (c) group defense via “mob capture” of large wasps near the nest entrance, (d) stinging behavior. In Japan, Ono et al. (1995) cited in Oldroyd and Wongsiri 2006:201 noted one additional colony defense behavior in response to attack from a hornet: recognition and removal of the marauding wasp pheromone before it has a chance to attract other hornets.

9.16.8 *Apis cerana* vs. *Apis mellifera*

There is an important early paper by Sakagami which outlines the competition and interaction of *A. mellifera* and *A. cerana* honeybee species in observations at mixed colonies (Sakagami 1959b). In Japan, the endemic *A. cerana* species was gradually replaced by *A. mellifera* with records of *A. cerana* extinction dating back to 1925 since *A. mellifera* were first introduced into that country in 1876. Japanese (alongside many other) apiarists preferred the introduced *A. mellifera* species given the ease with which they adapted to movable frames and their greater honey production. Sakagami summarizes that “in general, *A. cerana* is more tolerant and less aggressive than *A. mellifera*” p. 67. On p. 65 he writes that “with respect to interspecific conflict (in mixed colonies *A. mellifera*), usually took the dominant position in both aggressiveness and agility.” He notes the superiority of (*mellifera*) species in terms of their larger colony size, strong fighting capacity, and protection afforded by humans. Another difference between the two species was noted in terms of their foraging behavior. Citing Hachinoe 1954, Sakagami writes: “(*mellifera*) have a tendency to concentrate their effort on a major nectar source whilst (*cerana*) tend to forage from numerous minor sources.” He also makes the point that under natural conditions, the species would interbreed very rarely, if at all. Aligning with this assessment of less aggression of *A. cerana* compared to *A. mellifera* is evidence of their stinging behavior. Oldroyd and Wongsiri wrote that “*A. cerana* are more likely to retreat inside the nest than to sting on the approach of a mammal” (2006:161). In one experiment, although *A. cerana* do sometimes attack an intruder (an artificial mouse made of felt)—afterwards no stings were detected in the felt—whereas *A. mellifera* stings were extensive on the same target. Moreover, the sting of an *A. cerana* worker bee contains about half the quantity of stinging material (isopentyl acetate) compared to that of *A. mellifera* worker bee stings. However, it has been reported that *A. cerana* stings have an effect for considerably longer than *A. mellifera* stings (Wim de Jong May 24 2011, personal communication). Finally, *A. cerana* appears to have the least well developed barbs on the sting lancet compared to all other *Apis* species’ barbs (Ruttner 1988).

9.16.9 *Flight Patterns and Warming*

A. cerana bee flights are reported to be similar to fly flights in that they are rapid and unpredictable compared to *A. mellifera* flight patterns. There is also some unpublished evidence that *A. cerana* colonies in hives demonstrated 5.5 times as much flight activity relative to the number of bees in a colony compared to *A. mellifera* (Ruttner 1988). However, they tend not to fly far from their nests to forage; one source claims that this distance can be as far as 750 m but that 300 m is more typical (Punchihewa 1994).

Swarming activity associated with *A. cerana* reproduction is reported differently in different countries. In Japan, Tokuda recorded one, two, or three swarms per colony per year, while researchers in Pakistan recorded an average of eight swarms

per year (Ruttner 1988:144). Koeniger et al. (2010) report that in “tropical conditions swarms can survive and travel for several weeks. . . (however) longer periods of nectar scarcity or extended periods of rain will put the survival of a swarm at risk”. In Taiwan, Fen Tsung-Deh reported regular seasonal migration swarms by *A. cerana* between humid mountain areas and flatter areas (Fen Tsung-Deh 1952 in Ruttner 1988). In Australia, extensive field observation of the limited incursions to date indicate that there may be a difference in swarming behavior depending on whether they are (a) reproductive swarms (1–2 per year) or (b) absconding swarms (up to 7 per year). There may also be a difference in swarming behavior depending on whether *A. cerana* is in colonization (“bunker down after moving in”) or invasion mode (“up stakes and spreading out”) (de Jong, 24 May 2011, personal communication). Swarms of *A. cerana* who abscond (i.e., desert their nests) generally do so in response to a shortage in floral resources, an attack, or approach by predator/s or disease outbreak, e.g., from wax moths. Absconding behavior is reported differently in different countries—with more frequent reports of the behavior from Thailand and in temperate Japan and less frequent reports in South Asia (Ruttner 1988).

9.17 Nesting

As a species of cavity-dwelling bees, *A. cerana* colonies nest in hollow trees, caves, rock-clefts, walls, roof spaces, and rafters as well as nest cavities provided by birds, small mammals, and tree-dwelling reptile species. In Sri Lanka, it was thought that *A. cerana* require fully enclosed cavities to nest (Punchihewa 1994), however, this is not observed in other places. It is widely thought that *A. cerana* occupy smaller nest cavities than *A. mellifera* given their smaller physical size and colony size. *A. cerana* occupy smaller hives than *A. mellifera* when they are farmed and there is evidence that *A. cerana* colonies fail in standard size hives (Pandey 1977 cited in Ruttner 1988). However, in natural environments, *A. cerana* build nests in cavities with volumes as small as 4.5 L to as large as 97 L (Inouye et al. 1990 cited in Olroyd and Wongsiri 2006:153). In one study, *A. cerana* was found in a tree cavity with a diameter of 12 cm (Inouye et al. 1990 cited in Olroyd and Wongsiri 2006: 153). Unlike other cavity-nesting bees, cavity entrances of *A. cerana* nests vary widely (from 2–100 cm²) and are often found within 1–2 m off ground level (Inouye et al. 1990; Seeley et al. 1982 cited in Olroyd and Wongsiri 2006). *A. cerana* are one of the cavity-nesting species which thermoregulate nest temperatures. Where external ambient temperatures may vary between 12 and 36 °C, this bee species is able to maintain the brood nest temperature in the range of 33–35.5 °C. In particularly hot weather, *A. cerana* will use evaporative cooling mechanisms, collect water, and cluster outside the nest. In particularly cold weather, *A. cerana* have been observed to be using metabolic heat to warm brood nests. There is some evidence (well documented in Ruttner 1988) of *A. cerana* routinely “dismantling” old combs in nests in order to build new cells upon it. Arguably, this may contribute to more hygienic practices at the comb-site, but less as the old wax debris accumulate on the bottom of the hive and provide a suitable medium for wax moths (Ruttner 1988).

9.18 Mites

From extensive DNA matches, Denis Anderson of CSIRO demonstrated in a recent RIRDC workshop that parasitic bee mites are tightly matched in an evolutionary sense to their bee hosts (Rural Industries Research and Development Corporation and Horticulture Australia Limited 2010). Based upon molecular work conducted by Anderson and others (Anderson 1994; Anderson and Trueman 2000; Navajas et al. 2009), it is now accepted that host transfers from Asian species to *A. mellifera* are extremely rare. Transfers that have occurred took place in the past 40–100 years (Oidroyd and Wongsiri 2006:193). However, a recent CSIRO report for the Department of Agriculture, Fisheries and Forestry notes that a transfer occurred recently in 2008 (Anderson 2008). Species of *A. cerana*, *A. dorsata*, *A. laboriosa*, *A. florea*, and *A. adeniformis* are the primary host of three different genus of mites, *Tropilaelaps*, *Eugarroa*, and *Varroa*. Mites in the genus *Tropilaelaps* are parasites of the giant honeybees of Asia (*A. dorsata* and *A. laboriosa*) (Anderson and Morgan 2007). Some are occasionally observed inside *A. cerana* colonies in Asia (Ruttner 1988; Otis and Kralj 2001). However, except for one rare instance in Asia (Anderson and Morgan 2007), there is no other evidence that these reproduce on *A. cerana* brood (Otis and Kralj 2001). Mites in the *Eugarroa* genus are hosted by *A. florea* and *A. adeniformis*. *A. cerana* is host to three different kinds of *Varroa* mites—including *V. jacobsoni*, *V. underwoodi*, and *V. destructor* depending on the genotype of *A. cerana* (Anderson and Trueman 2000). So far only genotypes of *A. cerana* from northeast mainland Asia and the Japan region carry the forms of *Varroa destructor* which are so damaging to *A. mellifera* globally. The Java genotype of *A. cerana* carries mites that have long been known to be harmless to *A. mellifera*. However, in 2008 a harmful form of the mite was detected in Papua New Guinea (Anderson 2008). This mite did not accompany the Java genotype of *A. cerana* into the Solomon Islands (Anderson et al. 2010). The bee in the Solomon Islands carries a harmless form of the Java genotype of *V. jacobsoni*. *A. cerana* can effectively remove *V. jacobsoni* through grooming behavior consisting of self-cleaning, grooming dance, nestmate cleaning, and group cleaning. *A. cerana* worker bees can also rapidly and effectively remove *V. jacobsoni* mites from the brood. However, *A. mellifera* does not demonstrate such a high level of cleaning frequency and generally fail to remove significant numbers of mites from adult bees and brood (Peng et al. 1987).

9.19 Interactions Between Honeybees and Other Native Bees

The competition increases between *A. mellifera* and native bee species as many are of a similar size and require the same pollen and nectar resources for their progeny (Paini et al. 2005). This potential competition is explored further here. In Australia native flower-visiting insects (*Apoidea*) are smaller than the introduced honeybee and are not social (Michener 1970, 1979; Paton 1993). Their activity at flowers is usually concentrated in the middle of the day, when ambient temperatures are

highest. In an early reference to an unpublished study in Royal National Park near Sydney, Anderson notes that *A. mellifera* forage earlier and later in the day compared to native insects and that they outnumber endemic insect species (Anderson 1989). This difference in foraging activity gives the exotic honeybees an advantage in any competitive interaction, particularly if floral resources are present in the morning (Paton 1993). There is significant overlap in the floral resources used by honeybees and native bees indicating the potential for inter-species competition. In a Western Australian study of *A. mellifera* foraging behavior, of 51 species used by honeybees, 70 % of those were also foraged by native bees (Wills et al. 1990). This study indicates that native bees and *A. mellifera* coexist. However, authors commented that “other species of native bee which may have formerly inhabited this region may have already been displaced due to the effects of either direct or indirect competition with *A. mellifera*”.

9.19.1 Species Differences in Hybridization Efficiency

Sarma et al. (2007) studied the hybridization efficiency in four honeybee species and found that the number of complementary DNA (cDNA) spots (genes) hybridizing threshold fluorescence intensity was less for the three Asian species compared to *A. mellifera* (*A. mellifera* 5595, *A. cerana* 5202, *A. florea* 4556, *A. dorsata* 4535). Performances of the four species on the microarrays were comparable and in keeping with known differences and estimated approximate evolutionary distance. Thus, *A. cerana*, sharing a monophyletic origin with *A. mellifera* (Frisch 1927; Greiner et al. 2004), apparently displayed higher hybridization efficiency compared to the more distantly related *A. florea* and *A. dorsata*.

9.19.2 Similarities in Age-Dependent Differences in Brain Gene Expression

Sarma et al. (2007) found that of the genes found to be significantly regulated between foragers and one-day-olds in each of the species in the current study, 58–75 % also had been found to be significantly regulated between foragers and one-day-olds. All four species performed comparably on the *A. mellifera* cDNA microarrays and maintained consistent patterns of brain gene expression differences. This demonstrates the utility of the array for carrying out comparisons between *Apis* species.

9.19.3 Species Differences in Brain Gene Expression

A pairwise comparison between species showed that the comparison between *A. florea* and *A. mellifera* resulted in the greatest number of genes (114) showing FDO differences between any pair of species. This is perhaps a reflection of the

fact that of the four species compared, *A. florea* and *A. mellifera* show the most extreme differences in behavior and ecology. *A. florea* and *A. mellifera* are also among the most distantly related pairs of species in *Apis* (Greiner et al. 2004). However, the comparison between *A. cerana* and *A. dorsata* resulted in the fewest (18) number of genes showing F/DO differences between any pair of species and these two species also differ extensively in behavior and ecology. However, both *A. cerana* and *A. dorsata* are Asian species while *A. mellifera* is native to Africa and Europe (Land 1981).

However, these differences do not overlap with known ecological or physiological differences. For example, the differences do not reflect the fact that two of the species (*A. florea* and *A. dorsata*) are open-nesting while the other two are cavity-nesting; or that all four species differ in size with *A. dorsata* almost 2.5 fold larger than *A. florea* (Greiner 2006). There are indeed distinctive features of *A. dorsata* biology relative to the other three species, including migratory habits, defensive behavior, and its much larger size (Greiner 2006), but it is not known whether the differences we detected are related to these aspects.

9.19.4 Functional Classification of Differentially Expressed Genes

This is interesting because one of the most striking differences between the four species relates to differences in worker “tempo.” Measurements of colony attributes led Dyer and Seeley (Greiner et al. 2007) to conclude that open-nesting species (*A. florea* and *A. dorsata*) have a lower overall level of activity than do the cavity-nesting species (*A. mellifera* and *A. cerana*). It is reasonable to speculate that differences in colony activity levels are related to molecular processes associated with worker metabolism. Our results provide potential molecular correlates for these behavioral and ecological observations, and suggest that further analyses of genes in these categories would be particularly fruitful for understanding the ecology of genus *Apis*. Another category of genes were those implicated in circadian processes. Finding that genes related to circadian rhythms are overrepresented on the list of genes showing significant species differences in brain expression is notable from the perspective of honeybee dance language. Brockmann and Robinson (Oldroyd et al. 1992) discussed possible functional connections between the circadian system and the sun-compass system that is used by honeybees to communicate directional information during dance. The possibility of species differences in these systems is suggested by the fact that *A. mellifera*, *A. cerana* and *A. dorsata* dance on vertical combs and transpose sun-compass based information to gravity-based information, whereas *A. florea* dances on horizontal comb and does not make this transposition.

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Chapter 10

Dance Language

10.1 Introduction

Language is usually credited with being the major factor in making human society different from other higher animals. The fact that honeybees have a dance language that is unparalleled in the rest of the animal kingdom is therefore of great interest. That honeybees use dance language to recruit nest-mates to a food source has been known since Aristotle's time (Tautz 1996). When foraging honeybees find an attractive food source, they can perform a special communicative behavior called the dance language, which was first discovered by Karl von Frisch (1967a, b). More than half a century ago, Karl von Frisch put forth the astonishing hypothesis that honeybees (genus *Apis*) communicate the location of food and other resources through body movements he called dances. These dances, done by foragers on their return to the nest, had been described by many observers over several centuries and had long been assumed to play some role in communication about food. von Frisch's realization that dances carry spatial information was surely one of the major discoveries in behavioral biology in the twentieth century. Along with discoveries by other ethologists such as Lorenz and Tinbergen, the elucidation of the dance language opened our eyes to the sophistication and complexity of animal behavior and helped establish the study of behavior as a rigorous empirical science. Dances can be classified into three broad forms (von Frisch 1967a, b) which depend on the distance of the food source. For sources close to the colony, a simple round dance is performed. For larger distances, a sickle dance is performed. Finally, at the furthest distances from the nest, a waggle dance is performed. The waggle dance is the most sophisticated of these three forms as it encodes direction and distance of the food source (von Frisch 1967a, b; Dyer 2002; Tautz et al. 1996). Each iteration of the honeybee waggle dance consists of a straight waggle phase, whose duration indicates distance to the food source, and whose direction, relative to gravity encodes the direction of food relative to the sun's azimuth (von Frisch 1967a, b). Distance to a food source is gauged through the optic flow experienced on the outbound foraging trip (Esch et al. 2001; Srinivasan et al. 2000; Tautz et al. 2004). Recently, the waggle phase, instead of the entire circuit of the dance, was confirmed as a reliable indicator of the distance to the food source (Tautz et al. 1996; Michelsen et al. 1992; Seeley et al. 2000; Tautz and Sandeman

2003). By eavesdropping on this communication system, scientists have obtained a unique perspective into the perceptual world of insects (Chittka 2004; Sherman and Visscher 2002). The dance language of the honeybee is thought to have evolved from a more primitive form of communication, perhaps similar to that of extant bumblebees (Dornhaus and Chittka 1999). Moreover, various honeybee species may have evolved distinct ‘dialects’ during their long evolutionary history (Gould and Towne 1987; Dyer and Seeley 1991). Dance ‘dialect’ describes the distances at which foragers of each *Apis* subspecies make the transition between dance types. According to older published distance communication curves (Dyer and Seeley 1991; Lindauer 1956), *Apis florea* and *Apis mellifera carnica* display striking differences in their dialects. Further research has shown that the dance language could be influenced and affected by both genetic factors (Oldroyd et al. 1991; Rinderer and Beaman 1995; Johnson et al. 2002) and environmental parameters (Esch et al. 2001; Srinivasan et al. 2000; Tautz et al. 2004). Some comparative studies, based on these later findings, have shown that *Apis mellifera carnica* and *Apis florea* do not differ significantly from each other in the waggle phases, performed as a function of distance (Sen Sarma et al. 2004). However, these published waggle curves of different honeybee species were neither obtained from the same spatial route, nor at the same time. Thus, the question of whether these differences are real, or simply the result of flying through dissimilar visual environments, remains unanswered.

It is therefore necessary to obtain waggle curves of different species made to forage in the same location and at the same time. A mixed-species study on dance language communication is one possible way to investigate this issue. In general, individual honeybees from different species cannot be put together in one colony, because they have their own special odor, and are likely to attack and kill each other quickly (Winston 1987). This is the main obstacle to the study of social learning and communication between different species of *Apis*. Although honeybee workers and queens can be reared in any single-species colony, interspecific reciprocal introductions of female larvae between *Apis mellifera* and *Apis cerana* have usually failed (Oku and Ono 1990; Potichot et al. 1993), because of species-specific brood pheromones (Potichot et al. 1993; Ayasse and Paxton 2002) and/or differences in royal jelly (Takenaka and Takenaka 1996; Su et al. 2005). However, we are encouraged by the reports that young European *Apis mellifera* workers are accepted into Asian *Apis cerana* colonies (Atwal and Sharma 1967; Dhaliwal and Atwal 1970; Tan et al. 2006).

Su et al. (2008) studied dance language using video recordings in a mixed-species colony that was composed of the Asiatic bee *Apis cerana cerana*, and the European bee *Apis mellifera ligustica* and confirmed that *Apis cerana cerana* and *Apis mellifera ligustica* have significantly different dance dialects, even when made to forage in identical environments. When reared in the same colony, these two species are able to communicate with each other: *Apis cerana cerana* foragers could decode the dances of *Apis mellifera ligustica* to successfully locate an indicated food source. According to them, this is the first report of successful symbolic dance language.

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Honeybees are an important model for the study of animal communication because they use functionally referential communication to encode the distance and direction to a food source (von Frisch 1967a, b). As Karl von Frisch aptly stated, this system has been a “magic well” for behavioral ecologists and neuroethologists, continually providing important new insights (Giurfa 2007). Biologists know the most about one species, *Apis mellifera*, and it is therefore encouraging to see growing interest in other species of honeybees (Hepburn and Radloff 2011). The famous honeybee waggle dance and the variations that exist in different species have been recently well reviewed (Duangphakdee et al. 2011). Therefore, understanding how honeybee recruitment communication has evolved in different species is an area of interest. The exact number of honeybee species is debated, but many taxonomists recognize nine species: the dwarf honeybees (*A. andreniformis* and *A. florea*), the medium sized bees (*A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nigrocincta*, and *A. nuluensis*), and the giant honeybees (*A. dorsata* and *A. laboriosa*) (Radloff et al. 2011). In this chapter the focus will be on the Asian honeybees, primarily *A. florea*, *A. andreniformis*, *A. dorsata*, and *A. cerana*. It should be noted that *A. cerana* behaviors described in the literature may also apply to *A. koschevnikovi*, *A. nigrocincta*, and *A. nuluensis* given that recognition of these other species is relatively recent (Radloff et al. 2011).

10.2 Selective Pressures

Researchers have focused on two selective pressures influencing honeybee foraging: (1) the spatial distribution of food sources and (2) competition. Rich, clumped food sources can be valuable to recruit for (mass foraging), whereas more dispersed food sources may be better exploited by individual foragers that do not recruit to a specific location (Dornhaus 2002). In *A. mellifera*, colonies in temperate habitats (less clustered food) in which waggle dance location information was eliminated, gained the same weight as controls. However, colonies in an Asian tropical forest with more clumped food resources (determined from undisturbed waggle dances), gained significantly less weight when waggle dance location information was eliminated (Dornhaus and Chittka 2004). In addition, the role of resource spatial patchiness has been modeled, demonstrating the theoretical advantages of location communication for rich clumped food sources (Dornhaus et al. 2006).

How competition has shaped honeybee foraging communication is less well-understood. Honeybees are foraging generalists that collect nectar and pollen from a wide variety of species (Michener 1990). There is evidence for ecological niche overlap among Asian honeybees. For example, *A. florea* and *A. dorsata* visit many of the same floral species (Abrol 2011). Although *A. mellifera* is an introduced species in Asia, it is now in competition with native species, likely adding to competition that already existed among native species. For example, experimental removal of *A. mellifera* increased the number of *A. cerana* visiting four crop plants (Partap et al. 2000). Honeybees can reduce such competition by foraging at different times. The flowers of *Pterocarpus santalinus* open at midnight and are visited extensively by *A. dorsata* on moonlit nights from midnight until dawn. *Apis cerana* and *A. florea* visit only during early morning hours, competing with each other, but avoiding direct competition with *A. dorsata* (Rao and Raju 2002).

Honeybees can avoid competition by foraging on different plant species. *Apis florea* will visit species whose flowers provide low caloric rewards whereas *A. dorsata* prefers flowers with high caloric rewards. *Apis florea* visited several species such as *Daucus carota*, *Allium cepa*, and *Trigonella foenumgraecum* that were not visited by *A. dorsata*. However, specialized attraction may not always relate to caloric rewards. *Apis florea* was more attracted to *Brassica campestris* blossoms, even though these were less rewarding than *Cajanus cajan* flowers (Sihag and Rathi 1992). In addition, different floral nectar compositions are favored by different species (Abrol 2011). Species can also avoid competition by foraging under different environmental conditions and at different times of a day (Abrol 2011).

10.2.1 Conflict and Aggression

When multiple species simultaneously forage on the same resource, conflicts can occur. For example, *A. florea* exhibited aggression towards *A. mellifera* on flowers (Chahal et al. 1986). *More studies that examine aggression and conflict among native*

Asian species at natural food sources would be highly useful. However, research using artificial feeders and a mixture of native and introduced species (mainly *A. mellifera*) is still revealing. When *A. mellifera* and *A. cerana* were trained to separate sucrose feeders (50 % sucrose w/w) that were gradually brought closer together, aggression arose. Ultimately, *A. mellifera* were dominant and completely excluded *A. cerana* (Dhaliwal and Atwal 1970). At sugar water feeders, researchers found an aggression hierarchy among different Asian species. *Apis florea* was dominant over *A. cerana*, which was dominant over *A. dorsata*. *Apis florea* attacked *A. cerana* 13-fold more times than vice versa and *A. cerana* attacked *A. dorsata* 3-fold more times than vice versa. Conspecific aggression also occurred between *A. cerana* colonies (Koeniger and Vorwohl 1979). Similarly, Ruttner (1988a, b) found that *Apis dorsata* was attacked by *A. cerana*, not by *A. florea* or *A. mellifera*. At an artificial sucrose feeder, *A. florea* and *A. cerana* could dominate the feeder, excluding the larger *A. dorsata* and chasing off other species. In this study, *A. florea* and *A. cerana* foraged together without exhibiting aggression (Ruttner 1988a, b). Overall, small-bodied species exhibited more aggression and were dominant relative to larger-bodied species.

This hierarchy is counterintuitive because the larger individual usually beats the smaller in animal contests (Alcock 2001). However, in social insects, the ability of the colony to recruit large numbers of individuals plays a role. We can apply the concept of the contestant size to superorganism (colony) size, and not merely the size of an individual forager. A colony that can mass recruit a larger number of smaller-bodied workers can win against a fewer number of larger-bodied ones (Lichtenberg et al. 2010). Nonetheless, it is fascinating that in one-on-one contests a smaller bee can win over a larger one (Koeniger and Vorwohl 1979; Ruttner 1988a, b). This phenomenon is widely observed in stingless bees (Johnson 1974; Johnson and Hubbell 1974; Roubik 1978; Roubik 1981; Johnson 1983; Nagamitsu and Inoue 1997; Nieh et al. 2005). One explanation is that smaller species are more limited in flight range than larger species (Greenleaf et al. 2007). Therefore, the smaller-bodied species invest more effort and will take greater risks when fighting to defeat a larger-bodied species, which can abandon the contested patch to search for one further away (Koeniger and Vorwohl 1979).

A more complete understanding of social bee competition for floral resources should also include other bees, particularly the stingless bees, which can exhibit high levels of aggression and displace honeybees on natural food sources (Nagamitsu and Inoue 1997). *Apis koschevnikovi* and stingless bees visited honey water feeders placed at different heights in towers (Nagamitsu and Inoue 2005). In a separate experiment, stingless bees attacked *A. koschevnikovi* at honey water feeders, but *A. koschevnikovi* never exhibited aggression against stingless bees (Nagamitsu and Inoue 1997).

Honeybee nests provide rich resources. Inter- and intraspecific nest robbing can therefore occur, particularly when a colony is poorly defended. *Apis indica* and *A. mellifera* colonies can rob each other, depending on which colony is weak (Atwal and Dhaliwal 1969). In an apiary containing both *A. cerana* and *A. mellifera*, *A. cerana* initiated robbing of *A. mellifera* colonies more than vice versa, but *A. cerana* colonies were more likely to die during conflicts with *A. mellifera* colonies (Yang et al. 2011). Among native Asian species, robbing occurs between *A. dorsata* and *A. cerana* (Atwal and Dhaliwal 1969).

In some cases, robbing by *A. dorsata* can reduce the survival of *A. indica* colonies (Singh 1959). As with floral food competition, the extent of nest robbing among sympatric native *Apis* species is poorly understood. It would be useful to determine the patterns of interspecific nest robbing among different native species of Asian honeybees. Is there a hierarchical pattern, similar to a dominance hierarchy, in how species interact during nest robbing? The mechanisms that allow certain species to win fights against others remain poorly understood. In fights over a feeder, *A. mellifera* stung and killed *A. cerana*. (Dhaliwal and Atwal 1970). However, during intraspecific fights between *A. mellifera* foragers over an artificial feeder, I have rarely observed stinging. The primary mode of *A. mellifera* attack, as in stingless bees, involves biting (Nieh et al. 2005; Lichtenberg et al. 2010; Nieh 2010). Thus, species may adopt different attack strategies depending upon the competition. At nests, *A. florea* displaced *A. mellifera* on comb and fought with *A. mellifera* guards to rob their nest (Chahal et al. 1986). Although *A. mellifera* guards bit and pulled at the robbers on their legs, wings, and other body parts, *A. florea* often managed to escape.

How they did so, is unclear, but understanding their ability to escape may explain how they eventually won and successfully robbed the colony. *Apis florea* was able to sting and avoided being stung. Out of 124 dead *A. mellifera*, 14 % had been stung by *A. florea*, but 0 % of *A. florea* had been stung by *A. mellifera* (Chahal et al. 1986). Interestingly, once the colony had “submitted” there was relatively little resistance from the invaded *A. mellifera* (Chahal et al. 1986). This is reminiscent of the behavior of a stingless bee colony that initially fights and then retreats when invaded by the robber stingless bee, *Lestimelitta limao* (Sakagami et al. 1993). This phenomenon of “submission,” how it occurs, and what triggers it would be fascinating to study. From the perspective of the superorganism, how is the consensus to cease fighting achieved?

10.2.2 Signaling About Danger

In general, the effects of competition and fighting on food recruitment *communication* are not well understood. This is a part of the evolutionary story that remains to be fully explored. There is evidence for negative feedback signaling that counteracts the positive feedback produced by recruitment dances. During competition for a feeder, *A. mellifera* foragers that were attacked, returned to the nest and produced “stop signals.” The stop signal is a brief vibrational signal lasting around 150 ms (Kirchner 1993) at 380 Hz (Michelsen et al. 1986) and is frequently delivered by a sender butting her head into a recipient, most frequently waggle dancers (Nieh 1993). The stop signal or “brief piping signal” (Seeley and Tautz 2001; Thom et al. 2003) was originally described as a food begging call (Esch 1996; von Frisch 1967a, b). However, it does not elicit food exchange (Nieh 1993; Pastor and Seeley 2005). Instead, it causes waggle dancers to stop dancing and leave the nest (Kirchner 1993; Nieh 1993; Pastor and Seeley 2005). Bees produce stop signals under natural conditions and signal recipients waggle dancing for natural resources will reduce their waggle dancing

(Pastor and Seeley 2005). Natural and synthesized signals significantly increased waggle dancer departure (Nieh 1993).

Stop signal playbacks reduced waggle dance durations by 59 % and recruitment by 60 % (Kirchner 1993). In addition, deteriorating foraging conditions (crowding or increased feeder wait time) increase stop signal production (Thom et al. 2003; Lau and Nieh 2010). During competition for a rich food source, *A. mellifera* competitors attacked resident bees. Bees that were attacked (victims) increased the number of stop signals by 43-fold and sharply decreased (by 12.6-fold) the number of waggle dance circuits performed. Bees that were undisturbed (received and gave no attacks) and bees that attacked competitors continued to recruit and produced almost no stop signals (Nieh 2010). In addition, signalers targeted bees that smelled like the odor at their feeding location. The proximate causes of stop signal production can be further broken down into receiving physical aggression (biting) and detecting alarm pheromone. (Nieh 2010). There is a benefit to such a signal.

Each individual forager decides on whether and how much to recruit. However, one individual's decision to cease recruiting does not stop recruitment by other waggle dancers. By sending stop signals, she can inform foragers visiting the same location of adverse foraging conditions and give negative feedback to counteract waggle dancing by others. Broadly, collective foraging of the superorganism arises from the positive and negative feedback of multiple agents, with negative feedback cycles evidently providing greater precision and speed for labor reallocation. *Whether this occurs in other species of honeybees, particularly Asian species, is unknown. However, given the evidence for interspecific aggression at floral resources and during nest robbing, Asian species of honeybees may also have the stop signal. A comparative analysis could help us to understand how this signal functions and how it may have evolved.*

The stop signal is related to the experience of danger at a food source, and falls within a larger class of movement and vibrational signals related to danger. It would therefore be informative to examine other danger-related signals and see if they share common features. Several defensive behaviors (some of which may be signals) could be better understood. Defensive hissing signals are one example. Butler (1954) described a combined body shaking and hissing in *A. cerana*. *Apis mellifera cypria* produces high frequency hissing sounds when being attacked by the hornet, *Vespa orientalis* (Papachristoforou et al. 2008). Such hissing has not been reported in other subspecies of *A. mellifera*, although they are also attacked by wasps.

Apis florea workers produce a hissing sound during nest defense, combined with slight wing movements. These behaviors spread to nearby bees and may deter certain predators (Sarma et al. 2002). *It would be informative to learn if other Asian honeybee species produce hissing sounds when attacked by predators.*

The nests of open-nesting species are more exposed to predators than nest-cavity dwelling species. Open-nesting species have thus, evolved some interesting deterrents and signals. *Apis nuluensis* performs defensive shaking behaviors in response to hornet predators. This shaking recruited guards to the predator's location and also induced shaking in nearby bees (Koeniger et al. 1996). *Apis dorsata* produces shimmering patterns consisting of adjacent bees flipping their abdomens upwards.

This motion spreads throughout the comb surface in a wave-like pattern, and is stimulated by the presence of a predator, such as a wasp (Schmelzer and Kastberger 2009). Detailed video analyses show that wasps avoided the shimmering surface and were therefore prevented from directly capturing shimmering bees (Kastberger et al. 2008). There may be specialization in which workers perform the shimmering, since it is primarily triggered in the area where foragers arrive, dance, and then depart (Schmelzer and Kastberger 2009). Finally, drones are generally not thought to play a major role in colony defense. However, *A. cerana* drones reportedly participated in shimmering. Drone shimmering wing motions were less distinct than those of the workers, but were distinguishable (Sakagami 1960). Drone defensive behavior would be profitable to study because so little is known about what drones contribute to the colony, aside from their task of mating with virgin queens.

10.3 What Shapes the Precision of Location Communication?

What constrains the precision of the waggle dance? There is a continuous transition between round dancing and waggle dancing: from dances that provide no or small amounts of location information to dances that provide precise distance and direction information (von Frisch 1946; Gardner et al. 2008). Interestingly, there can be substantial variation in the waggle phase angle (divergence angle), but this generally decreases as the distance to the resource increases (Towne and Gould 1988). Two explanations have been proposed for this variance. *The adaptive hypothesis* states that this variance increases colony exploitation of resource patches by scattering recruits. Resources such as nectar or pollen typically occur in patches, not as points (unlike nest sites, Gould 1975; Weidenmuller and Seeley 1999). Therefore, the adaptive hypothesis predicts that waggle dances for nest sites should exhibit greater precision (less variance in waggle phase direction) than dances for food sources, at least for close distances. In the adaptive hypothesis, natural selection has shaped the divergence angle to improve colony foraging efficiency and nest migration. *The constraint hypothesis* states that this variance results from sensory and performance constraints. Under this hypothesis, the divergence angle is not a specific adaptation, shaped by natural selection in response to food source patchiness and house hunting. Rather, it arises from constraints on sensory mechanisms that bees use to orient their dances.

The constraint hypothesis proposes that bees are “doing the best they can” given the conditions they experience while dancing. For example, *A. mellifera* foragers with visual references during dancing had a smaller divergence angle than when they were deprived of visual references (Tanner and Visscher 2010). Since they normally have visual references while dancing, the open-nesting Asian honeybees provide an interesting natural test of the importance of visual constraints in the dance language. For example, in *A. florea*, there was a lull between noon and 1:00 p.m. in absconding dances, perhaps because of increased difficulty in determining the sun’s azimuth when it was at its highest point in the sky (Atwal and Dhaliwal 1969). In

Iran, *A. florea* built combs in which the horizontal section was clearly exposed to blue sky, perhaps to facilitate orientation (Tigari 1971).

Early experiments on open-nesting Asian species (*A. florea*, *A. cerana*, and *A. dorsata*) showed significantly smaller divergence angles than for *A. mellifera* at comparable distances (Towne and Gould 1988). However, Beekman et al. (2008) studied the spatial precision of *A. florea* waggle dances and found no significant differences between dance divergence angles for nest sites (point sources) and food sources (patches). Beekman et al. (2008) also found no significant difference between waggle phase durations (which provide distance information) for nest sites or food sources. Weidenmuller and Seeley (1999) studied *A. mellifera*, and likewise found no differences in waggle phase durations for nest sites or food sources.

However, Beekman et al. (2008) found an effect of distance on variance in waggle phase duration. In *A. florea*, variation in this distance information increased as the distance to the food source increased. Thus, as the distance to the resource increased, the distance information became less precise. This is different from what is reported for *A. mellifera*, where distance and direction information became more precise with increasing distance (Towne and Gould 1988). Beekman et al. (2008) thus suggested that directional information may be more important than distance information in *A. florea*. This matches what is reported for *A. dorsata* workers dancing on the surface of a swarm for nest sites (Dyer and Seeley 1994). These dancers exhibited consistent directionality but highly variable waggle phase durations (mean waggle phase duration of 42 dancers was 20.8 ± 16.5 s, with a range of 6.4–72 s). In contrast, dances for food sources exhibited less variance in dance circuit duration. *Given the evident low level of waggle dance distance information in A. florea (for food sources) and A. dorsata (for nest sites), it would be useful to examine the precision of distance communication in these species using feeder or nest site arrays.*

Interestingly, the same increase in imprecision with increasing distances (putatively conveyed through the duration of recruitment sound pulses) is reported in the stingless bees, *Melipona panamica*, *M. mandacaia*, and *M. bicolor* (Nieh and Roubik 1998; Nieh et al. 2003). Is there something about the habitat of tropical species that makes precise distance communication less important?

In terms of the constraint hypothesis, studies on the effect of dancing on a vertical comb vs. a horizontal comb would be informative, as Beekman et al. (2008) suggest. Such dances occur naturally in *A. florea*, which has a relatively flat portion of the comb above the plant branch holding the nest. For this species, horizontal dancers should rely only upon vision for orientation whereas vertical dancers could use vision and gravity. Careful experiments with this species may reveal if there are sensory constraints on waggle dance precision. *One predicts that in A. florea, for any given location, waggle dancers using multimodal orientation information (vision and gravity: on vertical comb surface) should exhibit different divergence angles from dancers using single-modal visual information (on the horizontal comb surface).*

10.4 Selection on Dance Dialects and Distance Coding

If one plots the duration of the waggle phase performed for any given distance, the duration of the waggle phase increases with increasing distance to the food source (von Frisch 1967a, b). The resulting curve is called the “dialect curve” or the “distance coding curve.” Has this dialect curve been shaped by the typical foraging range of a species? Some researchers have reported different codings of distance between honeybee species (Lindauer 1956; Puchihiwewa et al. 1985). These “dialects” may be shaped by species foraging ranges, based upon two assumptions. The first assumption is that steep dialect curves are more precise (providing a greater difference in signaling per unit increase in distance signaled). The second assumption is that the total steepness is constrained such that shorter maximum forager distances should correspond to steeper curves. Thus, species with different foraging ranges should have different distance codings. Early research reported that smaller bodied species (with smaller foraging ranges) had steeper dance dialect curves (Lindauer 1956; Boch 1957; Gould 1982; Puchihiwewa et al. 1985; Towne and Gould 1988). More recently, Su et al. (2008) compared the distance curves for *A. cerana* and *A. mellifera* and found significant slope differences. They reported that the distance curve slope for *A. cerana* was significantly steeper than that for *A. mellifera*. However, Dyer and Seeley (1991) found no significant differences between the distance coding “dialects” of the different Asian species of honeybees (*A. florea*, *A. cerana*, and *A. dorsata*) although, based upon decoding dances for natural food sources, there were significant differences in flight ranges. The larger-bodied species, *A. dorsata*, had the largest foraging range (95 % of dances were for distances estimated to be less than 3.8 km) compared to the smaller-bodied *A. florea* (1.3 km for the 95 % point) or smaller-bodied *A. cerana* (0.9 km for the 95 % point). The authors point out that observations over a variety of seasons are required for a more robust test of the dance dialect tuning hypothesis (Dyer and Seeley 1991). They conclude that their data may support the hypothesis of different dance dialects, but do not support the idea that dialects are strongly shaped by the maximum foraging distance of each respective species. They suggest that visual sensory constraints may explain why open-nesting bees (*A. dorsata* and *A. florea*) have relatively long maximum dance circuit durations in comparison with *A. mellifera* and *A. cerana*, which nest in cavities.

Instead of examining the slope of the dialect curve, one can measure the transition distance from round to waggle dancing. Towne and Gould (1988) found differences such that, *A. florea*, *A. dorsata*, and *A. cerana* transitioned to waggle dancing (called “oriented dances” in their paper) at much shorter distances (< 10 m) than *A. mellifera* (> 50 m). Whether these Asian species actually use this waggle dance information at such close distances and how precise recruits are would be useful to determine. However, Sarma et al. (2004) found no differences between the distances at which *A. mellifera carnica* and *A. florea* transitioned from round to waggle dances. Their paper introduces a useful technique of counting the number of circling runs and waggle phases. This avoids the problem of classifying the dance as either a round or a waggle dance, which can be difficult in borderline cases, and is a method that future studies may wish to use.

These differing results could arise if there is considerable intraspecific variation in the distance coding curves of each species. No studies, to my knowledge, have explicitly examined this by testing several colonies of the same species with the same procedures, but in different locations. Some intraspecific variation is expected. The distance at which a forager transitions from round to waggle dancing appears to be controlled by a single locus with multiple alleles (determined in a genetic backcross with different strains of *A. mellifera* (Rinderer and Beaman 1995; Johnson et al. 2002; Oldroyd and Wongsiri 2006). Recently, genomic changes have been detected when individuals were forced to shift from foraging at perceived short distances to perceived long distances by manipulating optic flow (Sarma et al. 2010). Another explanation for the dialect differences measured by some authors and the lack of strong dialect differences measured by Dyer and Seeley (1991), lies in the optic flow bees experienced in these different experiments.

Apis mellifera measures distance by measuring optic flow (Srinivasan et al. 1997, 2000; Esch et al. 2001). This likely is the same for all *Apis* species, because at least one species of stingless bee (*Melipona seminigra*) uses optic flow to measure distance (Hrncir et al. 2003), and one species of bumblebee (*Bombus impatiens*) can use optic flow during flight orientation (Dyhr and Higgins 2010). Given what we now know about the importance of optic flow in *A. mellifera* distance estimation, it would be useful to test Asian species for distance dialect differences using optic flow tunnels that allow for more controlled comparisons. Comparisons in natural habitats need to control for differences in optic flow that are perceived along the foraging route. For example, honeybees trained to fly through an optically dense habitat performed longer waggle phases than those trained to the same linear distance in a less optically dense habitat (Tautz 2008). In general, it would be useful to check whether the Asian honeybees (1) also use optic flow to measure distance and (2) how their odometer is calibrated and compares with what is known for *A. mellifera*. Also, more research on the vision of Asian honeybees would be beneficial, not only to better understand visual resolution and parameters that influence the perception of optic flow, but also to understand the fascinating phenomenon of night foraging. *Apis dorsata* eyes have large ocelli and are more sensitive to dim light than those of other *Apis* species (Somanathan et al. 2009). The bright yellow flowers of *Pterocarpus santalinus* open at midnight and are visited extensively by *A. dorsata* on moonlit nights (Rao and Raju 2002). However, the waggle dances of *A. dorsata* are evidently oriented towards the unseen sun, not the moon (Dyer 1985a). This raises the question of how these bees are able to avoid orienting towards a bright object in the sky (the moon) as a replacement for the sun, when *A. mellifera* generally accepts a bright object such as a lamp and uses this as a substitute “sun” (Gould 1975). Controlled experiments with *A. dorsata* colonies on moonless nights or in a darkened area and a light source of variable brightness could help to determine what levels of brightness this species finds acceptable as a substitute sun. It would be interesting to compare this threshold level of brightness with other species that do not forage at night.

Finally, do dance dialect differences actually matter? How is recruitment precision affected? Tan et al. (2009) created mixed colonies of *A. mellifera* and *A. cerana* and examined what happened when sender and receiver were the same or different species. They trained bees to feeder placed 130 m away within a linear array of four

feeders. There were differences in the number of bees recruited by intra- or inter-specific recruits. Surprisingly, precision of communication (where recruits showed up) was not significantly different between same- or mixed-species pairs (Tan et al. 2009) or in a similar experiment where foragers from mixed-species colonies were trained to a more distant, 500 m feeder array (Su et al. 2008).

In conclusion, much remains to be learned about why and how the honeybee waggle dance evolved and how, as a powerful force for positive feedback, it is countered by negative feedback communication. In testing the importance of various selective factors, it is important to recognize that the *value* of recruitment and recruitment information varies with multiple factors such as colony need, the availability and patchiness of food sources in the environment (Dornhaus and Chittka 2004), and the experiences of foragers (Grüter and Farina 2009). Given this range of factors, it is perhaps not surprising that experiments are sensitive to small changes in methodology and to the motivation of and private information possessed by recruits. One example of this complexity is shown in the different classifications of foragers: scouts, re-activated foragers, and experienced foragers (Biesmeijer and de Vries 2001). These categories reflect the motivation of individual bees to seek out certain types of information and food sources, and these likely arise from a motivational continuum. This should increase variance in the different behavioral outcomes. Recruiter motivation may also affect waggle dancing in more subtle ways, not just changing the number of dance circuits, but also varying how the dance is performed. Honeybees may not perform many waggle dances and can be very difficult to train to an artificial feeder (even a rich one) when there is abundant natural food. Whether the motivation to recruit also affects the accuracy of waggle dance information remains unclear, but it is known that waggle dances, at least in *A. mellifera*, can exhibit a wide range of variation and “excitement” from the classic “figure eight” to a forager that produces sporadic waggle phases interspersed by meandering around the nest (von Frisch 1967a, b). *Studies that examine this variation in detail would be illuminating, particularly by obtaining comparative data from the Asian honeybees.*

In closing, I would like to note that several phylogenetic trees have been suggested for *Apis*. These different trees influence our hypotheses on how the waggle dance evolved. It is tempting to see in the waggle dance, the ritualized path of a forager as she leaves the colony to collect food (waggle phase), returns to the nest to unload her collected food (return phase), and then returns to collect more food (waggle phase) again. The beautiful pattern of the waggle dance thus traces, in miniature, her foraging cycle (von Frisch 1967a, b). This analogy could be extended to the round dance, which occurs when the resource is close to the nest. Round dances can have a minor directional component (Gardner et al. 2008), but largely consist of circular “return phases.” We can call this scenario 1. In this scenario, the ancestral waggle dance pointed directly at the food source and was performed on a horizontal surface. Later, the complication of cavity-nesting (which provides no to very limited horizontal surfaces and limits visual orientation) led bees to dance on vertical surfaces, and thus the gravity-oriented vertical waggle dance evolved.

The real story may not be so elegant. The ability to dance on horizontal surfaces could be derived (scenario 2). In scenario 2, based upon molecular analyses and the cavity-nesting habits of all Euglossini, all Bombini, and most Meliponini, the

Apis ancestor was a *cavity nesting species*. All cavity nesting species build vertical honeycombs. All honeybees species waggle dance, and thus the ancestral *Apis* likely performed the waggle dance on a vertical comb (Koeniger et al. 2011). The small honeybees, *A. florea* and *A. andreniformis*, would then have evolved to nest on branches, where they have a horizontal component to their comb (the part above the branch) and a clear view of visual markers such as celestial cues to orient an evolutionary innovation, horizontal dances. Interestingly, *A. florea* seems unable to use gravity orientation for waggle dancing and lacks some of the gravity receptors found in other *Apis* species (Jander and Jander 1970). When *A. florea* foragers are forced to dance on a vertical surface, such as formed by a swarm, or in experiments in which the comb is rotated, they will dance such that their waggle run points at the goal, not with reference to gravity (Dyer 1985b). Instead, visual stimuli play an important role. Koeniger et al. (1982) found that *A. florea* dances on the horizontal surface of the comb were affected by an image of sky seen through a mirror.

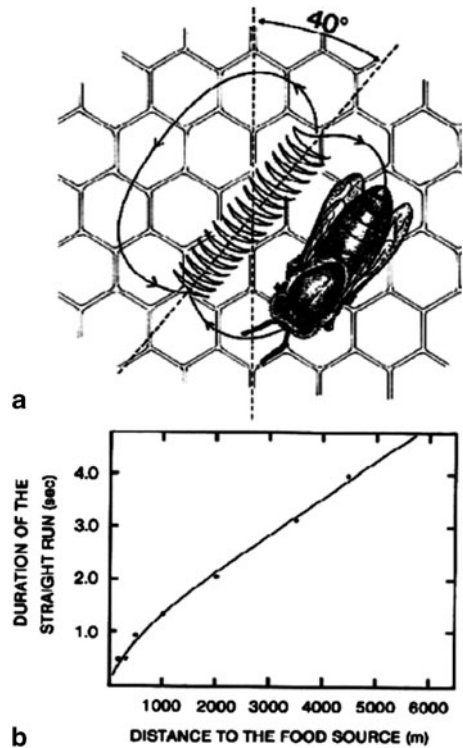
The cavity nester, *A. mellifera*, can also perform oriented dances on a horizontal surface when they have a sky view. It is unclear whether *A. dorsata* or *A. cerana* can perform oriented dances on a horizontal surface, although this would be interesting to determine. Surprisingly, the literature is, to the best of my knowledge, silent on this question. If *A. dorsata* and *A. cerana* can dance on horizontal surfaces, pointing in the direction of the resource, one parsimonious, though somewhat unsatisfactory answer, is that the ancestral *Apis* could dance on a horizontal surface and on a vertical surface (scenario 2). Alternatively, if we adopt scenario 1, *Apis* first evolved horizontal dances that pointed directly at the food source and then evolved vertical dances, *but never lost the ability to perform horizontal dances*. Thus, the existing species of *Apis* may not provide definite information about how the waggle dance evolved. However, detailed studies of what these species are capable of will still be valuable for understanding the communication of these fascinating organisms.

Dyer (2002) in an exhaustive review reported that honeybee foragers dance to communicate the spatial location of food and other resources to their nest-mates. This remarkable communication system has long served as an important model system for studying mechanisms and evolution of complex behavior. Dyer (2002) provides a broad synthesis of recent research on dance communication, concentrating on the areas that are currently the focus of active research. Specific issues considered are as follows: (a) the sensory and integrative mechanisms underlying the processing of spatial information in dance communication, (b) the role of dance communication in regulating the recruitment of workers to resources in the environment, (c) the evolution of the dance language, and (d) the adaptive fine-tuning of the dance for efficient spatial communication.

10.5 Spatial Communication Through Dance

In a typical instance of dance communication (von Frisch 1967a, b), a successful forager returns home from a rich food source and is greeted by other workers who, if she is carrying nectar, induce her to regurgitate her load to them. If this welcome

Fig. 10.1 Waggle dance of honeybees (Modified from Seeley 1985). During the flight to food or another resource, honeybees measure the direction (relative to the sun) and distance to the food. Direction is encoded in the orientation of the wagging run relative to gravity (or relative to the sun if celestial cues are visible during the dance). Distance is encoded in the duration of the wagging run. Different populations have different functions relating flight distance to wagging run duration. Other bees observing the dance use the spatial information it contains to fly to the general location of the food and odors carried by the dancer to pinpoint the actual resource. (Source: Dyer 2002)



is enthusiastic enough, the forager begins dancing on the vertical sheet of comb. The dance consists of a series of repeated wagging runs in which the bee moves in a particular direction along the comb while wagging her body from side to side.

During the wagging run she also emits a burst of sound by buzzing her wings. After each wagging run, the dancer circles around and realigns herself to begin the next wagging run. As the bee dances, she is encircled by 1–6 other bees that face toward the dancer and follow her movements. The dance followers observe several wagging runs and then leave the nest. Many of these eventually reach the same feeding place that the dancer had found or a feeding place close by. The orientation of the wagging run and its duration are highly correlated with the direction and distance that the forager has flown to the food (Fig. 10.1). Specifically, the angle of the wagging run relative to the upward direction on the comb correlates with the direction of flight relative to the sun and sun-linked patterns of polarized sky light. Dancers may also be oriented to these celestial cues directly if they can see them (e.g., when they are dancing on the surface of a reproductive swarm). The duration of the wagging run increases monotonically with flight distance, as can be observed in dances of bees trained to feeders at known flight distances. By placing arrays of feeders or baits in the environment, von Frisch found that recruits searched preferentially at baits near the one being visited by the dancer, suggesting that they had found their way there by using spatial information obtained from the dance.

von Frisch also described another form of the dance that he called a round dance because the bee circled repeatedly in place, occasionally changing the direction of turning. This type of dance is done by bees that have flown to locations near the nest. von Frisch suggested that round dances signal recruits to search near the nest, but they convey no information about direction. More recent research has revealed that many round dances actually contain directional information (Kirchner et al. 1998). Dancers produce sounds during round dances, when their bodies are aligned in the direction corresponding to that of the food. Thus, round dances may best be interpreted as waggle dances with short-distance signals. On the other hand, recruits that have followed round dances search in all directions near the nest (von Frisch 1967a, b), thus they may have difficulty obtaining directional information from such dances. von Frisch emphasized an important role for odors in the recruitment process. Specifically, he suggested that floral odors and other environmental chemicals cling to the body of the foragers and are detected by the dance followers. Foragers also release an attractive pheromone on their return to a familiar feeding place. The spatial information in the dance allows recruits to get only to the general vicinity of the food; odors allow them to pinpoint the resource indicated by the dancer (von Frisch 1967a, b).

A powerful source of odors can even lead recruits to ignore the spatial information in the dance and find food in locations other than the one being signaled. This effect of odors on recruitment is strong for nearby sources of food but weakens considerably as the distance to the food increases (Kirchner and Grasser 1998), which makes sense given the inherent imprecision of odors as a cue for food location.

The interplay of spatial information and odors is at the heart of the so-called dance language controversy, which arose in the 1960s as a result of the suggestion that odors were sufficient to explain recruitment of honeybees (Gould 1976; Wenner and Wells 1990). The proponents of this “olfactory search hypothesis” did not deny that dances contained spatial information (Wenner and Johnson 1967; Wenner and Wells 1990). They simply challenged the evidence that recruits use this information. In most of von Frisch’s recruitment experiments, spatial and olfactory information were confounded—the location being signaled would also contain the highest concentration of odors matching those carried by the dancer. However, some of von Frisch’s results were difficult to explain by the hypothesis that recruits use odors alone. For example, when deprived of orientation cues, dancers do disoriented waggle runs, and in this situation, recruits search in all directions rather than being biased toward the feeding place that the dancers are visiting (von Frisch 1967a, b). In spite of this evidence that odors alone are not sufficient to account for recruitment, the challenge to the dance language hypothesis was taken seriously and led to a number of clever experimental approaches that have attempted to separate the influences of spatial and olfactory information on recruitment (Esch et al. 2001; Gould 1976; Kirchner and Grasser 1998).

The consistent lesson from these studies is that odors carried by dancers are not sufficient to explain patterns of recruitment. Instead, essentially all experimental results can be accounted for by Frisch’s original hypothesis that dancers convey both spatial and olfactory information but can weight one more than the other depending on the strength or reliability of the information. The odor search hypothesis has not

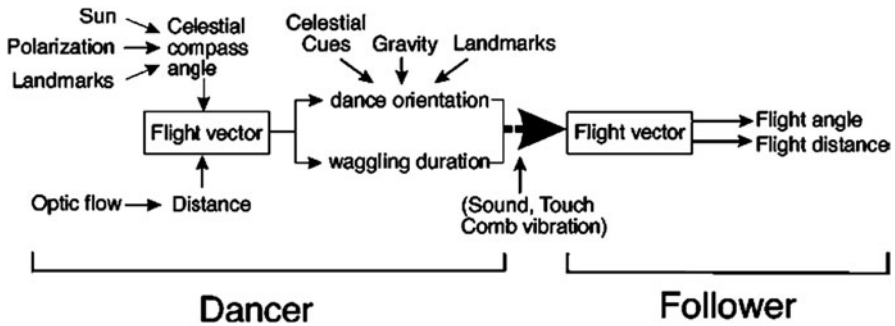


Fig. 10.2 Processing of spatial information in dance communication. See text for explanation. (Source: Dyer 2002)

been abandoned by its adherents (Rosin 1992; Wenner et al. 1991; Wenner and Wells 1990), but most researchers consider the dance language controversy to have been resolved beyond any reasonable doubt.

10.6 Spatial-Information Processing in Dance Communication

This section explores the sensory and integrative mechanisms that mediate the flow of spatial information through the dance communication system. Figure 10.2 shows the key information-processing steps. The forager must first measure the distance of the food and its direction relative to the sun (compensated for solar movement) to store in memory the vector pointing at the food. Bees can learn the direct route to the food even if they have flown a circuitous searching path to get there, a process called path integration. This vector that is the output of the path integration process is used for navigation on subsequent trips to the food, and it is also what the bee encodes in her waggle dance. To encode the path integration vector in the dance, the bee must measure her body orientation relative to environmental features available in the nest, which will often be different from those available during the preceding flight, and also translate her flight distance into the duration of wagging. The spatial information must now pass to other bees observing the dance. Their task is to measure the orientation and duration of the wagging run, using whatever sensory cues are available in the context of the dance, and to translate these measures into a vector corresponding to the direction and distance of the food. Using this vector to reach the food requires the bees to refer to sensory information available outside the nest, including the sun (which may have changed position since the dancer made her trip).

With this overview complete, I now turn to a consideration of the individual information-processing steps in this system.

10.6.1 *Measurement of Distance*

von Frisch suggested that bees determine their flight distance by measuring the expenditure of energy during the flight and that this measure weights energy expenditure on the outward flight more heavily than that on the homeward flight. In support of these conclusions are several observations (Esch and Burns 1996; von Frisch 1967a, b). (a) Bees loaded with lead weights signal greater flight distances than unloaded bees. (b) Bees signal greater flight distances in windy than calm conditions, and they signal greater distances if they have experienced a head wind on the outward flight than if they have experienced a tail wind. (c) Bees signal a greater distance if they have flown uphill to reach the food than if they have flown downhill. (d) Bees that have walked a short distance (3 m) to the food perform dances signaling a distance much greater than they have actually travelled, presumably because walking 3 m consumes more energy than flying 3 m.

von Frisch (1967a, b) also considered an alternative hypothesis, that bees measure distance by monitoring “optic flow”—the movement-induced streaming of visual texture across the visual field. Consistent with this hypothesis, bees that had flown to a feeder over a calm body of water (which provides weak optic flow) signaled a shorter distance than bees that had flown over land or over a wind-disturbed lake surface (either of which would provide a stronger optic-flow signal). However, although he recognized that optic flow could play some role, he regarded the energy hypothesis as more important.

Since 1990, Esch and other researchers have revisited this question in an extensive series of studies (Goller and Esch 1990; Ronacher et al. 2000; Srinivasan et al. 2000). In a striking turnabout, these studies have largely undermined the energy hypothesis and suggest that optic flow is the primary, if not only, source of odometric information for honeybees.

This conclusion is supported by a number of lines of evidence. First, bees trained to fly upward to a feeder 50 m above the ground signal a long distance if the feeder is on a building (which offers optic-flow cues during the ascent), but they signal a short distance if the feeder is suspended from a helium balloon in open country (which offers limited optic-flow cues) (Esch and Burns 1996). Raising the balloon higher actually shortens the distance signal, which is consistent with the optic-flow hypothesis but not the energy hypothesis. Second, bees can be trained to fly to food through a mesh-covered tunnel that has an artificial textured pattern on the walls and floor, so that optic flow can be controlled experimentally. Bees can learn the distance at which to expect food in such a tunnel (Srinivasan et al. 1996; Srinivasan et al. 1997; Srinivasan et al. 1999) tunnel (which should affect energy expenditure) had no effect on the ability to fly the distance they had learned, but manipulations of the optic-flow stimulus had a strong effect. Finally, observations of the dances done by bees that have flown through tunnels show that bees greatly overestimate the flight distance reported in their waggle dances if the tunnel walls are textured but produce a short-distance signal if the tunnel walls are untextured (Srinivasan et al. 2000). Also, dances by tunnel bees cause recruits to search in open country at a much greater distance than the foragers have actually flown to reach the food (Esch et al. 2001).

Esch and Burns have also pointed out that many of von Frisch's early experimental results, which he interpreted in support of the energy hypothesis, are consistent with the optic-flow hypothesis (Esch and Burns 1996). For example, wind and slope would affect energy expenditure, but they also affect the height that bees fly above the ground.

Bees fly closer to the ground in windy than in calm conditions and when heading up a slope rather than down a slope. Because nearby texture moves by more quickly than distant texture, the bee's height above the ground should strongly influence the optic-flow stimulus and hence the perception of distance.

The energy hypothesis has also been excluded as the odometer for desert ants (*Cataglyphis fortis*), which need to learn the distance to feeding places. On the other hand, ants can measure their travel distance when deprived of optic-flow cues (Ronacher et al. 2000), so they must obtain distance information from other sources, such as proprioceptive feedback as they walk.

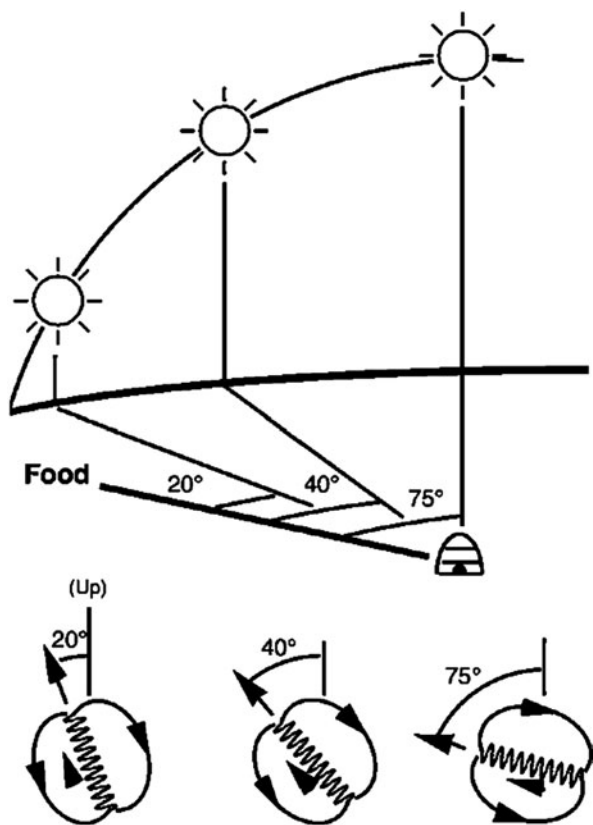
10.6.2 *Measurement of Direction: The Celestial Compass*

The sun has distinct advantages as a directional reference, including reliability, conspicuousness, and, because of its great distance, lack of susceptibility to motion parallax. Using the sun presents two major difficulties, however. First, it is sometimes obscured by clouds. Second, it moves. Observations of dances when the sun is behind clouds led von Frisch to realize that bees could also obtain compass information from the polarization patterns of light coming from blue sky (von Frisch 1967a, b; Wehner 1982; Wehner and Rosell 1985). These patterns, produced when the sun's light is scattered in the atmosphere, provide a directional reference that is essentially equivalent to that provided by the sun.

Because bees can orient their dances to patches of blue sky or to polarized light coming from artificial sources, the dance provides extraordinary opportunities to explore the mechanisms of polarization vision. One can manipulate the spectral content of an artificial patch of sky, its degree of polarization, its size, and its position relative to the bee, and observe the angle of dancing to infer how the animal perceives these celestial features. These experiments are done with bees dancing on a horizontal comb, so that gravity cannot be used for orientation. Coupled with investigations of the optical and neurophysiological mechanisms by which polarized light is detected, such behavioral studies have led a complete picture of how this source of celestial information is used for orientation (Wehner 1982; Wehner and Rosell 1985), a story that is beyond the scope of this review.

If the sky is completely obscured by clouds, then neither the sun nor polarized light is visible to bees (Brines 1978); however, overcast does not interfere with the ability of bees to find familiar sources of food and perform oriented dances. The explanation lies with landmarks. Bees can learn the path to food by reference to landmarks (von Frisch 1967a, b). Furthermore, they can learn positions of the sun relative to landmarks, so that when they need to perform a dance on a cloudy day,

Fig. 10.3 Dance communication as a window on the bee's ability to compensate for changes in the sun's azimuth (the sun's projection to the horizon). As the sun's azimuth shifts relative to the direction of the resource, the dance angle relative to gravity changes. By knowing the location of a resource (e.g., an artificial flower), an observer can assess the dancer's knowledge of the sun's changing position over time (Dyer and Dickinson 1994, 1996). (Source: Dyer 2002)



they can retrieve from memory the correct dance angle corresponding to the current foraging route (Dyer 1987; Dyer and Gould 1981).

It should be apparent that a memory of the sun (or of a dance angle based on the sun) would be useful only if it could be updated as the sun moves. von Frisch's studies of how bees use the sun for navigation were one of the first demonstrations of a time-compensated sun compass in any animal. These experiments involved training bees to find food in a particular compass direction and then assessing the accuracy of orientation relative to the sun after various time intervals during which the sun moved (von Frisch 1967a, b). More recently, the dances of returning foragers have been used to study the details of sun compensation. To indicate a fixed feeding place, dances oriented to gravity (which is also fixed) must shift to compensate for the changing angle between the sun's azimuth and the direction of the food (Fig. 10.3). More recently, the dances of returning foragers have been used to study the details of sun compensation. To indicate a fixed feeding place, dances oriented to gravity (which is also fixed) must shift to compensate for the changing angle between the sun's azimuth and the direction of the food (Fig. 10.3).

If one knows the location of the food, the waggle dance provides a readout of where the bee has determined the sun to be (Dyer and Dickinson 1996). This technique has

led to several insights into the sun compass of bees. (a) Observations of dances over several hours of overcast weather, when bees can see no celestial cues, have documented the accuracy with which experienced bees can compensate for the sun's movement by memory (Dyer and Seeley 1987). (b) Dances revealed evidence of nocturnal sun compensation by one of the Asian honeybees, *Apis dorsata*. Workers in this species undertake foraging trips on moonlit nights and perform waggle dances to nocturnal feeding places (Dyer 1985a, b). Although the moonlight is required for flight, the moon is not the reference for directional communication in these nocturnal dances. Instead, the bees signal directions relative to the extrapolated position of the sun, which they presumably find relative to landmarks visible by moonlight. (c) Observations of dances by bees that have experienced only a portion of the sun's course (e.g., during the 3 h preceding sunset) have provided insights into how bees learn the pattern of solar movement that is correct for the season and latitude at which they are active. We have known since the 1950s that bees learn the course of the sun during their first few days as foragers (Lindauer 1957, 1960; von Frisch 1967a, b) and that their knowledge is organized by reference to their endogenous circadian clock (Lindauer 1960; von Frisch 1967a, b). The long-standing mystery has been how they learn it from observations of the sun's position at different times of day.

Especially puzzling is the observation (Lindauer 1957, 1960) that bees can estimate the sun's position throughout the day even when they have previously seen only part of its course. Recent studies of this phenomenon (Dyer and Dickinson 1994, 1996) show that bees possess an innate template describing the general pattern of solar movement. This template automatically specifies that the sun rises opposite where it sets and crosses from one side of the sky to the other at midday. By default, the template describes an approximation of the sun's course, but it is updated through experience to represent the actual pattern of solar movement more accurately.

10.6.3 Dance Orientation: Coding Flight Direction into Dances

von Frisch showed that *A. mellifera* foragers could orient their dances either to gravity or to celestial cues. As far as we can tell, the orientation of dances to celestial cues involves the same mechanisms by which bees and other hymenopterans orient their foraging flights to celestial cues (Wehner 1982). In this sense the wagging run is a sort of pantomime of the flight (Wilson 1971). Orientation of the dance to gravity is mediated through proprioceptive bristle fields between the major body segments and the segments of the legs (von Frisch 1967a, b).

Until the 1980s, there was no reason to suppose that bees could communicate directions relative to any features other than celestial cues or gravity. If both of these references are eliminated by forcing bees to dance on a horizontal platform without a view of the sky, the dances are disoriented (von Frisch 1967a, b; Wehner 1982; Wehner and Rossel 1985). Magnetic cues provide no useful information for dance orientation (von Frisch 1967a, b), even though bees can orient their bodies to magnetic fields in other contexts (Frier et al. 1996; Collett and Baron 1994).

On the other hand, comparative studies have revealed that landmarks visible to the dancer can play a role in dance orientation. The role of landmarks was first identified in the Asian species *Apis florea* (Dyer 1985a, b). The nests of this species are quite different from those of *A. mellifera*. A single comb is suspended from a thin branch in dense vegetation and is protected from the elements only by a blanket of interlinked workers. Dances take place on the flattened surface above the supporting branch. The first studies of dance orientation in this species (Lindauer 1956) showed that there is no involvement of gravity but that celestial cues are used. Lindauer supposed that dancers were limited to the use of celestial cues and would be disoriented when these are blocked from view, for example, on a cloudy day. Following up on a later observation (Koeniger et al. 1982) that bees could remain oriented after celestial cues were blocked from view, I found that *A. florea* dancers can use landmarks seen from the nest (Dyer 1985a, b). I provided bees with artificial landmarks consisting of a stripe pattern partially surrounding the dance surface. After a period during which dancers could see celestial cues as well as the landmarks, I blocked the sky from view, without making it too dark to see the landmarks. The dances continued to be oriented toward the food, and when I rotated the landmarks to a new orientation, the dance angles changed with them. The typical *A. florea* nest in vegetation would provide a rich source of landmark references. Their ability to use such landmarks for dance orientation is probably essential for dance communication when celestial cues are blocked from view.

As important as landmarks may be in *A. florea*'s communication system, there was still little reason to suspect they would play a role for *A. mellifera*. Dancers in this species almost always have either gravity or celestial cues available as a reference, and several studies had shown that dancers are disoriented when both references are eliminated. However, no one had tested whether *A. mellifera* dancers could use landmarks if they were first given the opportunity to see them in conjunction with celestial cues (as was the case in the *A. florea* experiments). When we did this experiment (Capaldi and Dyer 1995), we found that *A. mellifera* is just as good at using landmarks visible during the dance as *A. florea* is. It remains to be seen what role this ability plays in nature.

10.6.4 Distance Signal: Coding Flight Distance into Dances

Several features of the waggle dance contain information about the distance the dancer has flown to food. von Frisch's standard measure was tempo, which he recorded as circuits per 15 s (von Frisch 1967a, b). Each circuit consists of a wagging run plus the return run that takes the bee back to begin the next wagging run. Tempo is easily measured by eye by recording the time period over which the dancer completes a given number of circuits. The same data can be used to compute average circuit duration, which is inversely related to the tempo. Tempo decreases with flight distance, whereas circuit duration increases. Other measures of the distance signal are hard to obtain in real time and instead must be obtained from video or audio recordings of

the dance. For example, the duration of the wagging run, the duration of the sound produced during the wagging run, and the number of waggles produced during the run all increase with flight distance. Within a population all of these variables are highly correlated, and thus provide essentially redundant information about distance.

The function by which flight distance is mapped onto the distance signal varies across different populations and species of *Apis*, producing so-called dialects (von Frisch 1967a, b). Interpopulation differences are also observed in the flight distance at which round dances give way to clearly directional waggle dances (von Frisch 1967a, b). Evidence that this dialect variation has a genetic basis comes from experiments in which workers from different *A. mellifera* races were reared together in the same colony and did not converge on a common dialect (von Frisch 1967a, b). The evidence for a genetic basis to distance dialects suggest that the tools of modern genetics may be applied in studying the mechanisms by which the visual signal from the odometer, which is recorded over a flight lasting perhaps several minutes, is translated into a duration of wagging lasting only a few seconds. One recent study provided evidence of a Mendelian pattern of inheritance for the flight distance corresponding to the transition from round dances to waggle dances (Rinderer and Beaman 1995). However, it is hard to interpret this in light of the evidence that a truly nondirectional round dance may not exist (Kirchner et al. 1998).

10.6.5 *Information Transfer from Dancer to Follower*

Given that the dance language is first and foremost a communication system, it is surprising how little is known about how the information in the dance passes to the follower bees (Michelsen 1999). Dances provide a rich variety of potential communicative stimuli, but it is unknown which stimuli the bees use. In considering the possibilities, note that the features of the dance that help followers find and stay with dancers need not be the features that carry the signal of spatial location. Three alternative sensory modalities have been suggested as the channel of information transfer in *A. mellifera*, none involving vision because dances of this species normally take place in complete darkness. These are (a) airborne sounds produced by the dancers' wings (and detected by the follower via the antennae), (b) vibrations of the substrate (detected via the subgenual organs), and (c) tactile cues (detected via the antennae and other sense organs on the head).

Evidence that airborne sounds play a role come from several observations, each of which is subject to some uncertainty. First, bees show spontaneous or conditioned behavioural responses to sounds in the frequency range typical of dance sounds, which suggests that they can hear these sounds (Towne 1995; Towne and Kirchner 1989). The relevance of these findings has been challenged on the grounds that the observed thresholds may be too high to allow bees to detect dance sounds (Michelsen 1999).

Second, recruitment rates are lower in several situations in which sounds are missing from the dance: (a) when dancers are spontaneously silent (as occasionally happens), (b) when the dancer's wings have been removed just prior to the dance, and

(c) when the dancer carries a mutant allele that causes diminutive wings (Kirchner 1997; Kirchner et al. 1998; Kirchner and Sommer 1992). However, it is possible that the motivation of dancers was reduced in each of these situations, affecting other relevant features of the dance. Or, perhaps sound serves to attract followers but carries no spatial information, so a silent dancer may have fewer followers, and hence reduced effectiveness in recruitment. Third, the production of airborne sound is necessary for a mechanical model bee to recruit bees to feeding places in the environment (Michelsen et al. 1992). Here again the possibility exists that the sound merely helps followers to stay oriented to the dancer but is not the channel through which spatial information flows. Furthermore, the recruitment efficiency of the model bee is low, suggesting that something beyond the presence of sounds and the correct pattern of body movement is needed for effective communication.

The hypothesis that substrate vibrations carry the dance signal is supported by various lines of evidence, all rather circumstantial. First, it has been argued that inefficiency of the mechanical model in recruiting bees is a consequence of the fact that the model is not in contact with the comb where the follower bees are standing, and hence cannot transmit vibrations to them (Tautz 1996). Second, bees appear to seek out open rather than wax-sealed brood cells when performing their dances, and recruitment efficiency is higher when dances take place on open cells than when they are on sealed comb (Tautz 1996). Sealed comb is presumed not to transmit vibrations as well as an open comb. The problem with this evidence is that the experiment did not control for the possibility that fewer recruits attend dances on sealed comb, or, alternatively, that recruits have difficulty following dances on sealed comb.

Such differences in recruit behavior might develop if, for example, prospective recruits avoided sealed comb because they are unlikely (normally) to find dancers there, or if they found it harder to maintain their footing while following dances on sealed comb. Third, it is possible to measure slight vibrations of the comb in the vicinity of a wagging bee (Nieh and Tautz 2000), although these vibrations are so weak they might be swamped by background noise during a normal dance.

Set against these lines of evidence supportive of a role for substrate vibrations is evidence that, whatever role they may play in some restricted circumstances, they are clearly not necessary for dance communication to occur. For example, in reproductive swarms of *A. mellifera* and on the exposed nests of the Asian honeybees *A. florea* and *A. dorsata*, dances take place on top of a curtain of interlinked worker bees (Lindauer 1956; Towne 1985). Because dancer and dance follower typically stand on different curtain bees, there is no path for the transmission of a vibratory signal. In such situations, a modality other than substrate vibration must be involved. A possible role for tactile cues is supported by the observation that there is substantial physical contact between dancers and dance followers during the wagging run. The challenge is to understand whether the tactile information is precise enough to account for the efficiency of recruitment (Rohrseitz and Tautz 1999). Other observations of the Asian honeybees make the picture even more complex.

Based on sound and video recordings of dancers, Towne (1985) reported an absence of dance sounds in two species that nest in the open (*A. florea* and *A. dorsata*), but intense dance sounds in the Asian hive bee *A. cerana*, which, like *A. mellifera*,

normally nests in enclosed cavities. Towne also observed striking differences in the postures of dancers in open- versus cavity-nesting bees. Both *A. mellifera* and *A. cerana* waggle their bodies side to side with their wings folded flat over the abdomen. *A. florea* and *A. dorsata*, by contrast, both add a dorsoventral oscillation to the wagging motion, so that the abdomen appears to flail wildly during the wagging run, and both species also hold their wings flared out to their sides. Towne suggested these postural features in the open-nesting species serve to make the dancer visually conspicuous to followers. Thus vision, as opposed to sound, may be an important modality for communication in open-nesting species. Subsequent studies using improved recording equipment confirmed that *A. florea* is indeed silent during its dances but that *A. dorsata* produces sounds similar to those of *A. mellifera* and *A. cerana*, although less intense (Dreller and Kirchner 1994; Kirchner and Dreller 1993). A role for sound in *A. dorsata*'s dances is further supported by the observation that dances are noisier when bees are dancing at night, when dances would be harder for followers to see (Dreller and Kirchner 1994). On the other hand, a close relative of *A. dorsata* from the Himalayas, *Apis laboriosa*, which appears never to fly at night, always dances silently (Kirchner et al. 1996).

Although these various observations complicate the pattern described by Towne, they are largely consistent with his basic idea that sounds play a role in dances that take place in low-light conditions and that dances that take place in the open may provide information through postural (visual) cues. It remains to be seen whether a cavity-nesting, sound-producing species such as *A. mellifera* can make use of visual cues when the dance takes place outdoors, for example on a swarm, and whether visual cues actually carry spatial information or if they merely serve to attract followers to the dancer.

Setting aside the question of which sensory channel carries the signal, a further issue concerns how bees translate the duration and orientation of the wagging run into a flight vector. This problem is perhaps straightforward in the case of the distance signal, where the duration of the signal (however it may be perceived) may directly translate into the magnitude of the flight vector. The problem is potentially more difficult in the case of the direction signal. At any given moment during the dance, followers are arrayed in various orientations relative to the dancer. Working out the compass direction being signaled in the dance would require the follower to measure her own orientation relative to both gravity and the dancer and then, in effect, transform her gravity angle into that of the dancer. The challenge of calculating this transformation would be further compounded by the difficulty of measuring relative body orientations using touch or sound. Theoretically, the bee could do this by exploiting spatial patterns in the sound field around the dancing bee (Michelsen 1999), but this hardly simplifies the problem. A pair of observations suggest the problem may be simpler than it would appear.

First, the choreography of dance following has the effect of frequently bringing dance followers behind the dancer and into alignment with her. If the follower could detect when she is behind a wagging dancer, then by measuring her own current body alignment at this point she is also measuring the dancer's wagging angle. Second, by using individually marked bees, Judd (1995) found that recruitment rates are higher

for follower bees that have had the opportunity to occupy the position behind the dancer than for followers that have observed dances from other angles. Thus, even if bees can measure relative body angles and calculate the necessary transformations, they may not be good at it.

10.6.6 Does the Waggle Dance Communicate Height?

The power of flight enables foragers to find food at various heights above the ground, and this realization led von Frisch to wonder whether there were any “words” in the honeybee dance language for height (von Frisch 1967a, b). He trained bees to find food by flying up or down tall cliffs or human-made structures. He found no evidence that the dances of these bees carried information about height nor that the recruits had obtained such information.

More recently, studies of an Asian cavity-nesting species, *Apis koschevnikovi*, have suggested that, given a choice between feeders at two different heights in a forest canopy, recruits will arrive preferentially at the one being indicated by dances (Roubik et al. 1999). However, the authors point out that this need not imply that height is being signaled in the dances. Instead, the recruits may head in the direction and distance indicated in the dances and then range vertically to locate the odors of their nest-mates at the food.

10.6.7 Dance Communication and Decision Making by Colonies

Dancing behavior is not an all-or-none stereotyped affair. Sometimes returning foragers do not dance at all but simply unload whatever they have collected and then return to the food source to collect more. If they do perform a dance, it may consist of just a few dance circuits or of a hundred circuits. This variation in the tendency to dance strongly affects where the colony’s recruits are sent. It has been known since the 1950s that the regulation of recruitment is not haphazard but results in the allocation of recruits to resources that are of greatest benefit to the colony. For example, bees are more likely to perform dances to nectar sources that are higher in concentration or closer, either of which would enhance the energetic profit to the colony. If the colony is heat-stressed, dances to sources of water become more common and more intense than dances to nectar (von Frisch 1967a, b).

Although a role for dances in the regulation of recruitment was recognized long ago, only in the past 20 years have the mechanisms underlying this regulation become clear. These mechanisms can be summarized by extending the information processing perspective developed in the previous section. However, whereas the previous section focused on spatial information, here the focus is on the processing of information about the value of alternative resources (Fig. 10.4). Furthermore, the flow of information is mediated not only by the forager’s experience in the environment but

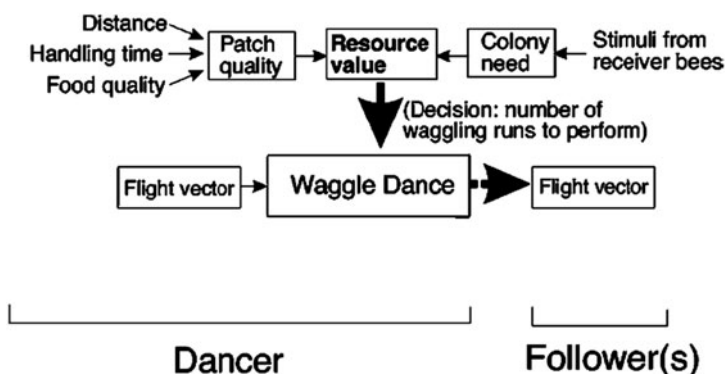


Fig. 10.4 Modulation of dance communication according to the value of the resource (a combination of intrinsic resource quality and the colony's need for the resource). The mechanisms for assessing resource quality and colony differ for different resources (e.g., nectar, pollen, water, nest sites). See text for details. (Source: Dyer 2002)

also by activities of nest-mates with whom she interacts. In fact, the social nature of the decision-making process leads us to consider the colony as the decision-making entity, faced with the problem of allocating a finite number of recruits.

These decision-making processes have been studied in four domains: nectar foraging, water-collection, pollen-foraging, and the selection of a new nest site by a reproductive or dispersing swarm. I give only a brief summary of the insights from this research because much of it was beautifully reviewed in Seeley's 1995 book (Seeley 1995).

A critical factor determining the level of recruitment for a particular resource is the number of dance circuits performed by bees that have discovered it. In the case of nectar sources, the decision of how many circuits a dancer should perform (if any) is based on the value of her resource relative to others currently available (Seeley 1986, 1995). This decision is partly influenced by information available only to the forager, including the distance to the flower patch, the handling time in the patch, and the sweetness of the nectar. Such cues indicate the intrinsic profitability of a patch but not its value relative to other patches. Because foragers do not directly compare patches, they cannot assess relative value directly. Instead they do so via a well-calibrated network of social feedback mechanisms that provide foragers information about the needs of the colony (Seeley 1985, 1995, 1998; Seeley and Buhrman 1999; Seeley et al. 1991).

The proximate indicator of colony-need (i.e., whether food of that quality merits additional recruitment) is the latency with which the forager is greeted by other bees and relieved of her crop load of nectar. Shorter latencies increase the probability of a forager's doing a large number of waggle dances to a patch she has found; longer latencies result in fewer wagging runs or none at all. The latency to be unloaded is affected by two critical factors. First, if a large amount of nectar is coming in from the environment, unloader bees tend to be occupied, in effect forcing foragers to

queue up to be unloaded. Second, if the colony is already full of honey, unloader bees take a long time to find an empty cell to deposit the nectar they have taken from foragers, thus they will be unavailable to receive incoming foragers.

The number of wagging runs done by a forager can be viewed as an indicator of the overall value of the resource (reflecting both its intrinsic profitability and colony need). This may not be the only signal of resource value provided by the dances. Many observers have noted that bees dancing to good patches seem more vigorous or lively than those dancing to poor patches (Alexander 1991; von Frisch 1967a, b), but it has been hard to quantify this subjective impression. In the case of round dances, Waddington et al. (Waddington 1982) documented various acoustic and locomotor correlates of food source quality, including a more rapid rate of circling, but such patterns were not obvious in waggle dances (p. 103). More recently, however, Seeley et al. (2000) reported faster return runs in waggle dances to more profitable food sources. Whether follower bees respond to these correlates of profitability is unknown, although it is possible that the liveliness of the dance serves to attract more followers and hence more recruits.

The immediate effect of long unloading times is to reduce the amount of dancing and recruitment to a given patch of flowers, hence limiting the rate of nectar intake from that resource. Clearly, however, it would be in the colony's interest to continue to harvest nectar from a highly profitable resource, if the capacity to handle the incoming nectar could be increased. Honeybee colonies have at least two feedback mechanisms that do this on different time scales. First, foragers that have experienced long unloading times can provide a signal to the colony of the need to increase the capacity to handle the incoming nectar. This signal is the so-called tremble dance, in which the forager meanders across the comb jerking her body and buzzing her wings in a characteristic way (Kirchner and Lindauer 1994; Seeley 1992). Workers that encounter a tremble dancer have a tendency to assume the role of unloader bee, hence decreasing the queuing time for incoming foragers. The second feedback mechanism, which works on a longer timescale, is the building of new comb, resulting in an increase in the capacity to store nectar (Seeley 1995). Although it is well established that the secretion of wax and the construction of new comb are initiated in times of high nectar flux (Ribbands 1953; Seeley 1995), it remains unclear what proximate cue triggers these processes (Seeley 1995).

The mechanisms regulating recruitment to resources other than nectar exhibit some differences from those I have summarized above, but they share some basic properties. First, the decision-making process is decentralized, with no direct comparison of alternative patches by any bee in the colony. Second, the decision of whether to dance is influenced by information obtained directly about the intrinsic quality of the resource and information obtained indirectly about the state of the colony or of the relative value of the resource. For details about the regulation of recruitment to these other resources, see the references listed (water: Seeley 1985, pollen: Camazine 1993; Camazine et al. 1998; Dreller et al. 1999, new nesting sites: Dyer 2000; Seeley 1985).

10.6.8 *Evolutionary Origin of the Dance Language*

Attempts to understand the evolutionary history of the dance language have relied on comparison of the communication systems of different living species of social bees. In many species of social Hymenoptera, including the social bees most closely related to honeybees, returning foragers interact with nest-mates and arouse them to search for food. In some species these interactions are reminiscent of honeybee dances (Esch 1967; Lindauer 1956; Nieh et al. 1999), and a consideration of these simpler dances will be useful for making inferences about the origin of the honeybee dance language. However, we begin with comparisons within the genus *Apis* itself in order to provide a detailed picture of the diversity of phenotypic characters that an evolutionary hypothesis must address.

10.6.9 *Origins: Insights from the Genus Apis*

Martin Lindauer's pioneering studies of the three *Apis* species that live in Sri Lanka (then Ceylon) led him to propose that the extant species of *Apis* exhibit a progression in the complexity of dance communication that corresponds to the phylogenetic development of the dance during *Apis* evolution (Lindauer 1956). The three species that Lindauer studied were the open-nesting "dwarf bee" *A. florea*, the "rock bee" *A. dorsata*, and the cavity-nesting Asian hive bee *A. cerana*. At that time these three species and *A. mellifera* were the only species recognized in the genus *Apis*; now other Asian species are recognized, but each of the new species is biologically similar to one of the species Lindauer studied, and so his comparisons captured the relevant diversity in behaviors related to dance communication. Lindauer suggested that the ancestral bee from which the dance language evolved was much like *A. florea*, building a single comb in the open and orienting its dances to celestial cues but lacking an ability to use gravity as a substitute for the sun. In the initial stages, this dance may have consisted merely of excited, disorganized movements that served merely to arouse nest-mates to search for food.

However, as these movements came to be oriented relative to celestial cues, and as nest-mates acquired the ability to bias their searching flights according to the orientation of the dances they observed, the communication system would have been heavily favored by natural selection. A later evolutionary stage is represented by rock bees such as *A. dorsata*, which Lindauer thought depended on a view of celestial cues while dancing on their exposed nests but nevertheless seemed to translate their solar flight angle into a dance angle relative to gravity. The most advanced stage is represented by cavity-nesting hive bees such as *A. cerana* and *A. mellifera*, which can use celestial cues if they are available but can also use gravity. In fact, the evolution of the ability to use gravity was supposed to have set the stage for the ancestor of hive bees to move into cavities. Lindauer's hypothesis has an element of circularity, in that it depends on a hypothesis about phylogenetic relationships based on the characters (nest architecture and characteristics of the dance) whose evolution he was trying to explain. Indeed, his suggestion that the ancestral *Apis*

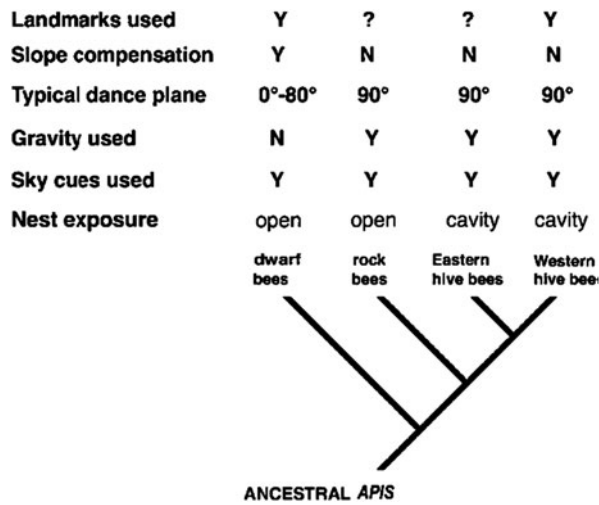


Fig. 10.5 Phylogenetic diversification of the waggle dance, as inferred from comparisons of directional communication and nesting behavior within the genus *Apis*. Phylogeny is based on molecular and morphological characters (Alexander 1991; Engel and Schultz 1997). The information on dance characters reflects both the original observations by Lindauer (1956) and newer work (Dyer 1985a, b, 1991a, b; Koeniger 1976; Koeniger et al. 1982). The cladogram shows only four taxa, but actually there are at least two species of dwarf bees, two species of rock bees, and four eastern hive bees in addition to the western hive bee *A. mellifera* (Smith 1991). (Source: Dyer 2002)

species nested in the open overlooks the fact that the construction of nests in the open is not observed in other social bees, hence appears to have been derived within the genus *Apis* (Koeniger 1976). Without independent support for Lindauer’s phylogenetic hypothesis, one could not exclude the hypothesis that cavity nesting was the ancestral condition among honeybees with respect to nest architecture and that the dance language evolved in an enclosed cavity rather than in the open.

In recent years, studies of morphological and molecular characters have provided such an independent phylogenetic hypothesis (Alexander 1991; Engel and Schultz 1997). These studies have vindicated Lindauer’s (1956) intuition that dwarf bees indeed diverged early on from a lineage that leads to the rock bees and then to the hive bees (Fig. 10.5). This would seem to support Lindauer’s contention that the dance language evolved on an exposed nest and that the return to enclosed cavities by the ancestor of hive bees occurred after the evolution of the ability to orient dances to gravity. However, parsimony is still equivocal on this point (1): (a) Open-nesting may have arisen in the ancestral *Apis* prior to the evolution of the dance language, followed by a reversion to cavity nesting in the ancestor to the hive bees (Lindauer’s hypothesis); or (b) open-nesting may have arisen independently in the dwarf bees and rock bees after the origin of the dance language.

Behavioral comparisons done in the past 15 years have further complicated the picture that emerged from Lindauer’s work. For example, although there remains no evidence that dwarf bees use gravity in their dances, rock bees can use gravity in the complete absence of celestial cues, just as in the hive bees (Dyer 1985a, b).

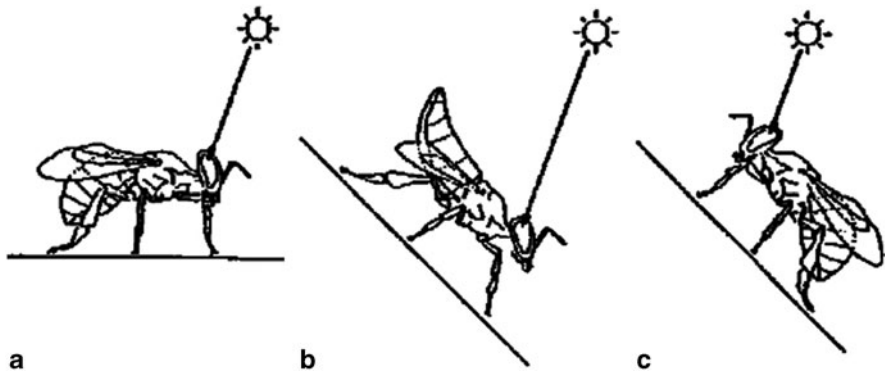


Fig. 10.6 Interspecific differences in the pattern of orientation to celestial cues by dancers that see the sun from a slope. **a** When dancing on a horizontal surface, both *A. mellifera* and the dwarf bee *A. florea* align their wagging runs so that they have the same view of the sun they observed during the flight (straight ahead in this example). **b** On a slope, *A. florea*, rotates its head to compensate for the slope, keeping its visual coordinates in a constant position relative to the horizon. **c** On a slope, the heads of hive bees are rotated along with their bodies so that the plane on which the bees are dancing defines the subjective horizon and the sun's apparent azimuth. In this example, to see the sun straight ahead the bee has to align her wagging runs uphill (Dyer 1991a). (Source: Dyer 2002)

Thus, among the extant *Apis* there exists a dichotomy, rather than an evolutionary progression, in the use of gravity.

Additional evidence of a phylogenetic dichotomy in the organization of dances emerged from a detailed comparison of the behavior of dancers on slopes. Lindauer's (1956) studies of the dwarf bee *A. florea* gave the impression that dances in these species are confined to a near-horizontal region atop the nest. Indeed, he reported that bees became confused when forced to dance on the vertical flanks of the nest. Later, however, I discovered that *A. florea* dancers frequently move down the steep slopes flanking the rounded dance area and can dance with a consistent orientation even if forced to dance on the vertical sides of the nest (Dyer 1985a, b). Although *A. florea* dancers exhibit a consistent pattern in their orientation in these experiments, their orientation differed strikingly from that seen when *A. mellifera* dancers orient to celestial cues on steep slopes. If an *A. mellifera* dancer is exposed to the sun or a bright artificial light source, it will orient its wagging runs to the apparent azimuth of this source relative to the plane on which the dance takes place. In *A. florea*, by contrast, dancers use the actual horizon as the reference for determining azimuth, even if they are dancing on a rather steep slope. The difference arises because *A. florea* dancers counter-rotate their heads to compensate for slope, so that their visual field remains in a stable relationship relative to the actual horizon. In *A. mellifera*, by contrast, the head rotates with the body as the dancer walks onto steeper slopes, so the plane on which the dancer is standing defines the apparent horizon to which celestial cues are referenced (Dyer 1985a, b). Figure 10.6 shows an example of this difference.

These observations suggest that there are two types of waggle dance with respect to the communication of direction: the type seen in the dwarf bee lineage (as exemplified

by *A. florea*) and the type seen in the lineage that includes rock bees and hive bees (as exemplified by *A. mellifera*). This pattern complicates the problem of making inferences about the evolution of the dance language from comparisons within the genus *Apis* because it provides no way of assessing under parsimony which type is ancestral and which is derived. One way of obtaining additional clues would be to find evidence of hidden similarities between these two lineages. Traits that are present in all taxa, even if not routinely expressed, can be interpreted as plesiomorphic for the genus. An example is the ability to use landmarks as references for dance orientation. As mentioned earlier, this ability was first described for *A. florea* (Dyer 1985a, b) and was presumed to be absent in other species; hence at first it appeared to be one of a suite of characters unique to the dwarf bee lineage. However, later studies uncovered evidence of this ability in *A. mellifera* (Capaldi and Dyer 1995). Thus, this trait may be a universal property of honeybee dances, supporting the conclusion that it was part of ancestral dance language (Dyer 1991a, b). Conceivably, the same sort of evidence could be adduced for other components of the dance language.

10.6.10 Origins: Insights from Other Social Bees

Comparisons of the extant *Apis* species have uncovered a number of ambiguities concerning the polarity of key evolutionary transitions. The standard way that phylogenetic methods resolve such ambiguities within a group is to study character states in outgroups that exhibit homologous traits. One complication in doing this with the dance language has been the difficulty in determining the phylogenetic relationships among honeybees (tribe *Apini*) and their closest relatives. These relatives include the stingless bees (Meliponini), which like the honeybees are highly eusocial; the bumble bees (Bombini), which are primitively eusocial; and the orchid bees (Euglossini), which are solitary (Michener 2000). Interest in the phylogenetic relationships among these four taxa has been driven primarily by the question of whether eusociality arose once in a common ancestor of honeybees and stingless bees, or independently in these taxa (Cameron 1993; Chavarria and Carpenter 1994). For the purpose of understanding the evolution of the dance language, however, the value of a phylogenetic hypothesis is to indicate which taxon is the sister to the honeybees, and hence is the best choice for outgroup comparisons. In spite of some lines of evidence placing bumble bees or orchid bees as the sister taxon to honeybees (Cameron 1993), a total evidence phylogenetic analysis favors the stingless bees (Chavarria and Carpenter 1994).

Even if we work from the assumption that stingless bees are the relevant group for outgroup comparisons, we still face the problem of identifying behavioral traits that we might use to polarize evolutionary changes in the dance language of honeybees. The difficulty is that the communicative interactions in stingless bees show few obvious similarities to the features of the honeybee dance language that allow for accurate spatial communication.

Most species of stingless bees studied to date exhibit a behavior reminiscent of the dances of honeybees (Hrnčir et al. 2000; Jarau et al. 2000; Lindauer 1956; Nieh 1998). Returning foragers run among their nest-mates buzzing their wings and dispensing samples of the food that they have brought back. These dances arouse other bees, which move out of the nest and fly in search of food. In some species, these dances play a role in spatial communication, leading to the recruitment of nest-mates to locations near where the foragers have been feeding rather than at control feeders offering food in other locations. In some cases, this spatial communication is mediated entirely by odor marks deposited by the knowledgeable foragers on their way back to the food (Lindauer 1956). On the other hand, a role for odor trails has been excluded in some species of *Melipona* by training bees to locations across water and showing that recruits still preferentially arrive at the station visited by the dancers (Jarau et al. 2000; Nieh et al. 1999). Experiments of this kind have suggested that the bees' dances might communicate not only direction and distance but also height.

It remains unclear just how this information might be communicated, but sounds made by the dancers offer an intriguing possibility. In *Melipona panamica*, the durations of sound bursts produced by the dancer were found to correlate with flight distance, and other sounds made by the forager as she was unloaded correlated with the height she flew (Nieh and Roubik 1998). No obvious feature of the dance correlated with flight direction, leading to the speculation that direction is signaled by foragers performing exaggerated flights toward the food as they depart on their next trip to the food (Esch 1967; Nieh 1999).

As intriguing as these speculations are, it is important to bear in mind that the evidence for spatial communication by *Melipona* dances is weak. In one species that shows spatial biases in recruitment, *M. quadrifasciata*, detailed measurements of dance features found no evidence of spatial information in the dance (Hrnčir et al. 2000).

Furthermore, in no species have odors been excluded as the factor biasing the searching of recruits toward the location visited by the dancer. It is true that odor trails deposited by foragers have been eliminated as an explanation in some cases, but it is possible that recruits can orient to other feeding-site odors carried by the dancer. Thus, all of the same concerns raised against von Frisch's recruitment experiments during the dance language controversy arise here, especially in light of the fact that stingless bee recruitment experiments take place over relatively short distances. Even in *A. mellifera*, recruitment over short distances is strongly influenced by odor, independent of the availability of spatial information (Kirchner and Grasser 1998). Even though the question of whether the dances of stingless bees signal spatial information remains unresolved, these dances support at least one conclusion concerning the evolution of dance communication. Dance behavior—an intensive interaction at the nest between returning foragers and their nest-mates—arose prior to the origin of the genus *Apis*. Hence, these dance precursors must have arisen in bees that nested in an enclosed cavity, where bees would be deprived of celestial orientation cues and would be forced to provide vibratory or tactile signals. Beyond this, however, it is impossible to determine on the basis of these comparisons whether an *Apis*-like dance, with precise directional and distance communication, could have arisen in an enclosed cavity.

The lesson of this section thus far is that comparisons of overt dance-like behavior by returning foragers provide little guidance regarding the polarity of key transitions in the evolution of the honeybee dance. An alternative approach might be to consider the polarity of behavioral elements that may play a role in dance behavior and that may be expressed in a noncommunicative context in outgroup taxa that lack dances. One example is the ability to orient to gravity, which in most insects is expressed as simple geotaxis and plays a role in escape responses. Comparisons of geotaxis in various bee taxa revealed that the *Apis* species that use gravity in their dances exhibit a phylogenetically derived form of geotaxis, whereas *A. florea*, which does not use gravity in its dances, resembles outgroup taxa in its geotactic response on slopes (Jander and Jander 1970). This is consistent with Lindauer's hypothesis that *A. florea*'s inability to orient dances to gravity is a primitive condition. This interpretation is not without ambiguity (Dyer 1991a, b; Horn 1973; Koeniger 1976), but this example still stands as a nice illustration of how to make outgroup comparisons when the outgroup taxa do not exhibit the trait in question.

10.6.11 Adaptive Design of Dances for Efficient Spatial Communication

Viewing the dance language as the product of evolution invites us to consider ways in which it may have been optimized by selection for its function of communicating spatial information. As in the case of the historical question of how the dance originated, studies of this functional question have relied on comparative studies, here examining how the dance varies with the goal being indicated or how it varies across different populations or species of honeybees. One possible example of this, discussed earlier, is the tendency for species that dance in darkness to produce sounds during the wagging run and those that dance in the open to have exaggerated postures that may enhance visual information transfer. Here I consider three additional aspects of dance communication that have been studied as possible instances of the adaptive fine-tuning of the dance language.

10.6.12 Distance Dialects

Boch's (1957) and Lindauer's (1956) discoveries of population and species differences in the slope of the distance-dialect function led von Frisch (1967a, b) and others (Dyer and Seeley 1991; Gould 1976) to speculate about the possible adaptive significance of these differences. von Frisch's hypothesis was that the slope of the dialect function evolved under two major influences. First, he suggested that steeper slopes allowed for more precise communication, in that a given amount of error in producing or reading the signal would translate into a smaller amount of error in the distances searched by recruits. Second, he suggested that the steepness of dialect

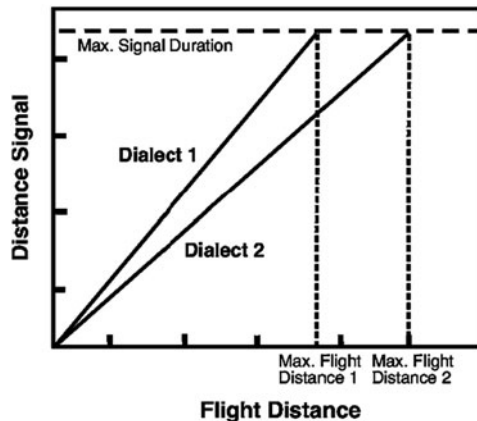


Fig. 10.7 Summary of von Frisch's hypothesis that distance dialects are tuned to ensure maximum precision over the flight range of the bees (where flight range is determined independently by ecological factors or body size). Steeper dialect functions are assumed to be more precise (see text), but a function that is too steep would produce wagging runs that may be hard for other bees to follow. Assuming a common upper limit on distance-signal duration, then populations with a shorter flight range should evolve steeper dialect functions (Dyer and Seeley 1991; von Frisch 1967a, b). (Source: Dyer 2002)

functions would be limited by a constraint on how long the distance signal could be for dances indicating the limits of the colony's flight range. If the function were too steep, wagging runs for distant sites might be so long that the recruits would have difficulty staying with each wagging run, let alone sampling several of them.

This hypothesis makes the prediction that the steepness of the dialect curve and the maximum typical flight range of bees in the population should be inversely correlated (Fig. 10.7). In populations where flight range is constrained (by body size and ecological factors) to a shorter typical distance, dialect curves should evolve to be steeper. Testing this prediction requires a comparison of flight range and dialect curves in the same population. It is relatively easy to measure the dialect curve—one trains the bees to a series of known distances and records the dances, although a potential difficulty is that the shape of the curve may vary among foraging routes depending on visual features of the terrain that influence the optic flow experienced by foragers (Esch et al. 2001). Another challenge is to get an accurate picture of foraging range. Earlier studies used the unreliable technique of training bees as far as they would fly to an artificial feeder (Lindauer 1956) or compared flight ranges in disturbed habitats (Punchihewa et al. 1985).

More recently, Dyer and Seeley (1991), in a study of *A. florea*, *A. dorsata*, and *A. cerana*, used the technique of "forage mapping" (Visscher and Seeley 1982), whereby one observes dances to infer how far bees have flown to natural feeding sites. Our evidence appears at first to undermine the adaptive-tuning hypothesis. We found that the dialect curves of these three species in Thailand were virtually identical in their slope. This contrasts with the situation in Sri Lanka, where Lindauer (1956) and others (Punchihewa et al. 1985) found dialect differences among these species.

Given the similarities among the dialects in Thailand, the adaptive-tuning hypothesis predicts that the maximum flight distances in this region would be similar for bees in the same habitat. We found, by contrast, that *A. cerana* had a short flight range (approximately 2 km) compared with *A. florea* (11 km) and *A. dorsata* (12 km).

Although we rejected the adaptive-tuning hypothesis as an explanation for the dialects of different species, an interesting pattern emerged in the forage-mapping data that may support an altered version of the hypothesis. von Frisch supposed that different populations of honeybees are subject to a common constraint on the maximum feasible duration of the distance signal. We found, by contrast, that the longest distance signals seen in dances of *A. florea* and *A. dorsata* (one fourth of 30 s) were about three times the maximum signal duration observed in *A. cerana* in Thailand or in earlier studies of *A. mellifera* in North America (Visscher and Seeley 1982) or Africa (Schneider and McNally 1993). Thus, there is not a universal upper limit on signal duration. Instead, there may be a different constraint for the open-nesting species than for the cavity-nesting species, perhaps related to the differing roles of vision and sound in dance following. If so, then a fair test of the adaptive-tuning hypothesis would require comparisons between dialect and flight range only among open-nesting species or among cavity-nesting species. Considering only data collected in relatively undisturbed habitat, the predictions of the adaptive-tuning hypothesis, controlled for nest architecture, are supported (Dyer and Seeley 1991).

10.6.13 Tuned Error in the Divergence Angle

A peculiar feature of the waggle dance of *A. mellifera* noticed by von Frisch (1967a, b) was that wagging runs are consistently aligned in the direction of the food only when the flight distance is fairly long (i.e., several hundred meters). In dances to short distances, successive wagging runs diverge from each other, alternately missing to the right and left of the true direction. von Frisch described a steady decrease in this divergence angle as flight distance increased. Haldane and Spurway (1954), in their pioneering paper applying information theory to the communicative signals of animals, proposed a functional explanation for the relationship between divergence angle and flight distance. They suggested that divergent dances tend to spread out recruits so that they would more rapidly discover the full extent of a floral resource distributed in a patch rather than as a point source. Furthermore, the decrease in the divergence angle with flight distance was explained by the fact that patches of a given size would subtend a smaller angle at the nest when at greater distances. Thus, the divergence angle was interpreted as a source of useful error, optimally tuned to the spatial distributions of resources in the environment.

There was little evidence bearing on this intriguing hypothesis until Towne and Gould (1988) took up the problem in a wide-ranging experimental and comparative study. One prediction of the hypothesis is that the absolute error in the distribution of recruits attracted to baits in the field should be roughly constant as the searching distance increased, as a result of the decrease in the signal error at greater flight distances. Towne found that the searching error actually increased with searching

distance, but did so gradually. The diameter of the search area at 700 m was roughly twice as large as that of the search area at 100 m. However, the angle subtended at the nest by the search area at 700 m ($15 \pm$) was only one fifth that at 100 m ($75 \pm$), in support of the hypothesis that recruits were guided by more precise information at greater flight distances.

In another experiment, Towne (1985) compared dances to feeding places with dances (by bees on swarms) to nest boxes. Because nests are always point sources and never diffuse patches, the tuned-error hypothesis predicts a smaller divergence angle in dances to nests than in dances to food. Towne found no differences in the dances to these different types of resources. More recently, Weidenmueller and Seeley (1999) found the difference predicted by the tuned-error hypothesis: small divergence angles for nest sites and larger divergence angles for food at the same distance. They suggest that Towne failed to find the difference because the bees he observed dancing to a nest box had first been trained to a feeder placed on the nest box, and thus may have had difficulty detecting the change in behavioral context. When they trained bees to a nest box using food rather than letting them discover it as house-hunting scouts, they too observed no difference between food dances and nest-site dances.

Towne (Towne 1985) also provided comparative evidence that supports the hypothesis that the spatial precision of the waggle dance is tuned to the spatial distribution of resources. He studied three tropical species of *Apis* (*A. cerana*, *A. florea*, and *A. dorsata*), which he reasoned would be confronted with flower patches that would typically be small (e.g., single flowering trees) in comparison to flower patches in temperate zones. The tropical bees would therefore be more heavily penalized by a large divergence angle at an equivalent flight distance. As predicted by the tuned-error hypothesis, all three species showed divergence angle only at short flight distances. Their divergence angles were reduced to less than $5 \pm$ for flights of only 150 m. Races of *A. mellifera* from temperate regions, by contrast, show divergence angles of $20\text{--}25 \pm$ at equivalent flight distances. In short, both experimental and comparative data provide support for the hypothesis that spatial precision of the dance, and the dispersion of search activity by recruits, is adaptively tuned in a way that corresponds to the spatial distributions of resources being communicated.

10.6.14 Migration Dances

Recent studies of two tropical honeybees have uncovered evidence of a different style of dance communication in the indication of migratory direction. One of these species is the African hive bee *A. mellifera scutellata* (Schneider and McNally 1994), and the other is the Asian rock bee *A. dorsata* (Dyer and Seeley 1994). In both species, colonies make seasonal migrations of tens or hundreds of kilometers (Koeniger and Koeniger 1980) in response to regional shifts in rainfall and the availability of floral resources. Migration, and the role that dance communication plays in colony movement, is different from what is seen in the two other main types of colony movement—reproductive swarming and emergency absconding—when the

colony is under threat of predation or natural disaster (Dyer 2000). Swarms and absconding colonies move temporarily to a resting spot near the natal nest and from there send out scouts to find new nesting sites nearby. The scouts return to perform dances indicating the locations of candidate nest sites (Lindauer 1955; Seeley and Buhrman 1999).

Migrating colonies of both *A. dorsata* and *A. mellifera scutellata* depart directly from the natal nest on a long flight in the migratory direction. In both species (Schneider and McNally 1994), the dance has been modified to play a role in organizing the initial move. The migratory dances begin a few days before colony movement, and by the time the colony takes off, dozens of bees perform dances. These dances signal the compass direction in which the colony ultimately departs, and hence resemble nest-site dances on reproductive or absconding swarms. They differ in interesting ways, however. First, whereas dances on swarms contain accurate information about both the direction and the distance of the new nest site, the migratory dances are accurate only with respect to direction. Migratory dances are much more variable with respect to the distance signal than are dances to discrete resources. Furthermore, the average duration of the wagging run is extremely long, corresponding to flight distances of many tens or hundreds of kilometers.

Such distances are well beyond the flight distances that bees could be expected to travel from the nest. Finally, observations in the early morning showed that migration dances begin before any bees leave the nest, suggesting that the bees do not base the signal on spatial information gathered on a trip just preceding the dance (Dyer and Seeley 1994). These dances could be based on information gathered during flights on previous days, but this behavior still differs dramatically from that observed in dances to discrete resources. In short, the migration dances reflect the emergence of a colony-wide consensus about the direction that the colony should travel, but they do not signal actual locations sampled by the dancers. Nothing is known about how migratory directions are chosen or how the consensus is reached.

Although the structure of the dance language is very similar among species of honeybees (Lindauer 1956), communication of the distance component of the message varies both intraspecifically (Steche 1957; Boch 1957) and interspecifically (Lindauer 1956). Dance dialects means the distances at which foragers of each *Apis* species make the transition between the round and waggle dance types and different distances encoded in the waggle runs if the waggle dances were performed. According to Lindauer's communication curve, *A. florea* and *A. mellifera carnica* display striking differences; however, Dyer and Seeley (1991a), reported that three Asiatic honeybee species, *A. florea*, *A. dorsata*, *A. cerana*, show very similar dance curves.

Some researchers have shown that the dance language could be influenced and affected by genetic factors (Oldroyd et al. 1991; Rinderer and Beaman 1995; Johnson et al. 2002), while others have shown environmental parameters to also have a strong impact on foragers' dances (Srinivasan et al. 2000; Esch et al. 2001). Combining these findings, the dialects of honeybee species are rather complicated. For example, when based on the latter factor, Sarma et al. (2004) found that *A. florea* and *A. mellifera carnica* showed quite similar dances. Unfortunately, all of these findings were not based on the same spatial route and same time parameters. Using

mixed-species colonies of *A. mellifera ligustica* and *A. mellifera carnica*, Steche (1957) and Boch (1957) showed that the dance language includes dialects' such that, foragers of both races of honeybees were recruited by each other's dances; but with consistent misinterpretations of the distance component of dances. Similarly, variations in the waggle dance among races of *A. cerana* have also been reported (Sasaki et al. 1993) but remain equivocal (Lindauer 1956; Dyer and Seeley 1991a).

10.6.14.1 *Apis cerana cerana* and *Apis mellifera ligustica* Possess Distinct 'Dialects' of the Waggle Dance

Su et al. (2008) reported that under normal conditions, i.e., in single-species colonies, *Apis cerana cerana* foragers consistently had a much greater waggle duration for a given distance than did *Apis mellifera ligustica* foragers, as has been previously reported (Gould and Towne 1987). Further, the waggle durations of dancers from both species increased in a linear manner with increasing distance. The slope of the distance-waggle duration curve for *Apis cerana cerana* in the single-species colony was significantly steeper than that of *Apis mellifera ligustica* bees in the single-species colony. The slope for *Apis cerana cerana* in the mixed-species colony was also significantly steeper than that of the *Apis mellifera ligustica* bees in the same colony. Moreover, at the 100, 200, 300, and 400 m positions, foragers of a particular species generally displayed similar waggle durations, regardless of whether they were from a single-species or mixed-species colony. The waggle duration results show that there really are dialect differences between *Apis cerana cerana* and *Apis mellifera ligustica*.

10.6.14.2 *Apis cerana cerana* Foragers can be Recruited by *Apis mellifera ligustica* Dancers in a Mixed-Species Hive

Su et al. (2008) found that that *Apis cerana cerana* foragers could follow the dances of *Apis mellifera ligustica*, and use this information to successfully forage at particular feeder positions in a mixed-species colony. A critical issue in these experiments was the fact that only one feeder was present at a time. Thus, one could argue that when bees of one species were found at the right location, as advertised by the other species, it was not because they were able to decode the 'foreign' distance information, but simply because they had no alternative choice. In order to address this possible criticism, we performed further recruitment experiments in which recruited bees were offered different feeders at different locations simultaneously.

10.6.14.3 *Apis cerana cerana* is Able to Acquire Food-Related Information from *Apis mellifera ligustica* in the Mixed Hive

Su et al. (2008) reported that trophallaxis was observed frequently between *Apis cerana cerana* and *Apis mellifera ligustica* workers which also shows that *Apis cerana*

cerana and *Apis mellifera ligustica* could communicate food-related information with each other by transferring food reward.

10.6.14.4 The Number of *Apis cerana cerana* Followers is Greater than that of *Apis mellifera ligustica* Followers in the Mixed-Species Colony

Our observations of dance-following behavior in the mixed-species colony indicate that *Apis cerana cerana* bees were much more likely to follow a dancer than *Apis mellifera ligustica* bees (Su et al. 2008). However, individuals of each species generally displayed a similar likelihood of following dancers from both species. This is the first report of the successful establishment of a mixed-species honeybee colony, with individuals of *Apis cerana cerana* and *Apis mellifera ligustica* cohabiting, foraging and carrying out normal hive functions, for the greater part harmoniously, for over 50 days. Several cross-species interactions, such as dance following, trophallaxis and queen tending were observed during this period, indicating that ours was a normally functioning hive. We believe that this is an important breakthrough in the study of honeybees, and that such mixed-species hives will open exciting new avenues of research into various aspects of this social insect's biology. We studied details of the dance communication (dance angle, waggle duration, and recruitment success) of *Apis cerana cerana* and *Apis mellifera ligustica* in the mixed-species hive. The dance angles were not significantly different between *Apis cerana cerana* and *Apis mellifera ligustica* in the mixed-species hive, which means that both dance dialects indicated the same food source direction. However, the distance-dependent waggle durations were significantly different between *Apis cerana cerana* and *Apis mellifera ligustica* honeybees, regardless of whether they were in a pure colony or the mixed-species colony. The dialect differences of honeybee species are therefore encoded in the difference in waggle duration. Environmental variables, such as wind velocity, temperature, and the surrounding landscape can be ruled out, as all bees were made to forage along the same flight path, and all dances for a given experiment, whose waggle durations were analyzed, were recorded within a short period of time.

10.7 Modulation of Dance Communication in Response to Nectar Toxicity

The ability to communicate the location of food sources to nestmates means that the discovery of a profitable food source by just one forager is sufficient to allow a colony to exploit that source rapidly (Seeley and Visscher 1988; Beekman and Ratnieks 2000; Beekman and Lew 2008). But what if the food source is toxic? Alkaloids commonly associated with herbivore defense are present in the nectar of some plant species, so that their nectar is toxic or repellent to most floral visitors (Adler 2000). During periods when there are limited floral resources, honeybee colonies may be obliged to exploit food sources that they would normally ignore (London-Shafir et al. 2003; Liu and Fu 2004; Nicolson and Human 2008). For example, in southern China,

during the summer, the Asian hive bee, *Apis cerana*, is forced to forage on the toxic nectar of a perennial vine, *Tripterygium hypoglaucum*, also known as the thunder god vine, because there are limited alternative food sources. Nectar produced by *T. hypoglaucum* contains a terpenoid known as triptolide, which is mildly toxic to bees (Tan et al. 2007). Given a choice, bees prefer nontoxic honey to that of *T. hypoglaucum* (Tan et al. 2007). In addition, honeybee foragers can learn to associate odors with toxic effects (Wright et al. 2010).

Tan et al. (2012) in a very interesting observation observed that foragers of the Asian hive bees, *Apis cerana* collecting *T. hypoglaucum* honey modulate their recruitment dances depending on the availability of alternatives. Because some nectars, such as that of the thunder god vine, *Tripterygium hypoglaucum*, contain alkaloids that are mildly toxic to honeybees, *Apis* spp. Given a choice, foragers prefer nontoxic honey to that of *T. hypoglaucum*, but only if there are no alternative nectar sources. When alternative nectar sources were available, dancers decreased the frequency of waggle dances and increased the frequency of tremble dances. Furthermore, the waggle dances were less precise than usual. These changes are likely to reduce recruitment. By contrast, when there were no alternative nectar sources available, foragers collecting *T. hypoglaucum* honey performed near-normal dances. Because dance behavior is dependent on the alternative food sources available, changes in the bees' behavior is probably not due to the nectar's toxicity per se. They concluded that modulation of in-hive communication serves to protect the colony from death caused by the collection of high quantities of toxic food while preventing starvation when no other food is available. Tan et al. (2012) found that the bees clearly indicated that they preferred common vetch honey syrup over *T. hypoglaucum* syrup, with lower return rates, lower probability of waggle dances, increased probability of tremble dances, reduced number of waggles in the waggle phase, and longer return phases in dances for *T. hypoglaucum*. However, when natural nectar was scarce, the proportion of foragers that danced for *T. hypoglaucum* approached that for common vetch, strongly suggesting that the bees modulated their dance behavior depending on the availability of alternative nectar sources. Nectar produced by *T. hypoglaucum* contains a terpenoid known as triptolide, which is mildly toxic to bees (Tan et al. 2007). Given a choice, bees prefer nontoxic honey to that of *T. hypoglaucum* (Tan et al. 2007). In addition, honeybee foragers can learn to associate odors with toxic effects (Wright et al. 2010).

10.8 Future Directions

Karl von Frisch once described the honeybees and their dance language as a “magic well” of scientific discovery, remarking that “the more you draw from it the more there is to draw.” This well continues to yield new insights and new questions. Here I want to point to two additional questions that have received relatively little attention in this review and that in my view represent especially fruitful lines of future inquiry.

First, the ability of bees to code navigational information in waggle dances and to translate dances into a vector that can be used to guide a searching flight suggest that

bees can solve an interesting computational problem. At the most general level, this is a mapping problem: how to translate spatial coordinates of a resource, measured visually over several minutes of flight, into the motor commands necessary to control the orientation of the wagging run (relative to gravity) and its duration. Follower bees must solve this same mapping problem in reverse. It remains puzzling how all this happens. To some extent the mappings involve innate transformations of sensory data. This is true of the mapping of flight distance to wagging run duration (Esch and Burns 1996; Srinivasan et al. 2000) or of the mapping of solar flight angle into a gravity angle (von Frisch 1967a, b). Some of the mappings are learned, however. For example, bees learn to compensate correctly for the sun's movement, and thus must have some way of learning the function (or fine-tuning an approximate innate function) that describes the progression of the solar azimuth over time relative to fixed features of the terrain (Dyer and Dickinson 1994, 1996). What sorts of neural events might underlie these various mapping processes is unknown. Given the importance of such processes in navigation by other animals, their study in bees (where the dance provides a window onto the processes) may produce insights of general interest to neurobiologists.

The second area of research is the use of the dance as a tool for studying the foraging ecology of bees. One can infer the flight distances travelled by bees by measuring the distance signals in randomly sampled dances to natural foraging sites and then using the dialect function to decode the distance traveled. By measuring the directions indicated in the dances, one can compile a two-dimensional map of the colony's foraging activity over a given period of time. This forage mapping procedure has already been used to study shifts in a colony's use of different foraging patches over time (Schneider and McNally 1993; Visscher and Seeley 1982) and to compare the foraging activities of different colonies in the same habitat (Dyer and Seeley 1991; Schneider and Hall 1997; Waddington et al. 1994). The full potential of this technique has yet to be realized. Of special interest are studies in natural habitats where honeybees are important indigenous pollinators—especially the African and Asian tropical forest. Given that tropical forest plants are predominantly insect pollinated, understanding the foraging ecology of pollinators is relevant to an understanding of forest community ecology. Among the most important questions to answer about pollinator behavior is flight distance, which directly affects dispersal distances of pollen. This question is easily answered through forage mapping. When combined with other information, such as the composition of the diet (determined by sampling pollen brought back by foragers), the rate of foraging from colonies, and the sizes and densities of colonies in the environment, it may be possible to obtain a detailed picture of the dynamics of pollen flow in the environment.

These two lines of future research illustrate how deep Karl von Frisch's magic well really is, allowing us to address fundamental questions about the sensory and computational mechanisms underlying behavior, as well as questions about community ecology. The use of the dance to study questions about sensory mechanisms, adaptive design, and evolution of behavior also remain active areas of research. Thus, we are far from exhausting the capacity of this amazing behavior to teach us about the workings of the natural world.

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Chapter 11

Foraging

11.1 Introduction

The habitat of most animals provides resources of different types that are essential for the species' survival, but these may not necessarily be close together. Accordingly, these animals have to forage to bridge some temporal or spatial distance to locate the essential resources (Schoener 1971). Foraging can be defined as the search for and acquisition of food. It is one of the most consistent and demanding tasks for any given living organism related to its survivability. The data on foraging ability and foraging behaviour are, therefore, necessary to the understanding of population dynamics and community structure of bees as well as to develop conservation strategies. The foraging behaviour of social insects is especially interesting because individuals do not forage to meet their own nutritional needs; rather they forage to meet the needs of the colony. Foraging labour is also divided such that some individuals forage for nectar, some for pollen, some individuals return to the nest carrying both nectar and pollen, and a small proportion of the foraging force return with water. The foraging requires information of locations, distances between locations, and the availability of food. The honeybees that consistently return to the same location are called central-place foragers in which every foraging trip consists of distinct 'outward' and 'homeward paths'.

Flowering plants and honeybees have a special relationship in which both are benefited from each other, as honeybees get food and in turn facilitate their pollination process. Honeybees visit flowers to collect pollen and nectar. Pollens are the principal source of protein, fats, vitamins and minerals, which are essential for honeybee growth and development, repairing of worn out tissue and stimulating the development of hypopharyngeal glands. Nectar consists of carbohydrate compounds mainly sucrose, fructose and glucose portion of the honeybee's food and is the raw material of honey (Jones and Yates 1991).

Foraging behaviour of bees is a complex phenomenon which depends upon several factors. Besides, physical features of flowers such as colour, shape and odour, the diversity of environmental factors such as temperature, humidity, light, solar

radiation, time of the day and nectar flow decisively shapes the behaviour of pollinating insects thereby influencing the cross-pollination and the production of the crop (Visscher and Seelay 1982; Corbet et al. 1993). The activity spectra of both flowers and pollinators coincide on daily and yearly rhythms (Faegri and van der Pijl 1978). The bees and flowers make a unique mutually compatible system, the functions of which are affected by a set of common environmental factors in a manner that functional activity of bees and flowers synchronize.

To achieve it, there are obvious interactions between biotic and abiotic factors such as temperature, light intensity, solar radiation, wind velocity, rains, dew drops, time of the day, distance to food sources, availability of resources, abundance of nectar and concentration of sugar in nectar. Diel pattern of bee visits are related to the changes in the quality and quantity of floral rewards as well as the direct influence environmental factors. Hence, the complex interactions of biophysical parameters and food rewards determine foraging success (Heinrich 1975a). Bertsch (1983) stated that close dependence between pollinating insects and their flowers can be better understood, if the functions of flowers, their adaptations and the needs of flower visitors are known, which would consequently lead to the management of a particular group of pollinators in a set of ecological conditions.

Floral patterns attracting pollinators and other specialised mechanisms resulting, therefore, seem to have evolved in relation to the sensory development of the bees which in the course of development gradually perfected their own sensory faculties and physical efficiency to identify colour, odour, iconic numerals and symmetry of flowers. Differentiation of nectaries accompanying floral evolution became responsible to trophallactic activities of progressively evolving contemporary pollinators which directed specific changes in their award-getting capabilities (Leppik 1977). Thus, co-evolutionary adaptations by the vast variety of angiosperms passing through several niches of time have chiefly been attributed to the mutually interrelated modifications amongst flower and their pollinators. Obviously, rewards system offered by the flowers plays a determinant role besides interplay of other factors which participate in attracting floral visitors. In this connection, flower visitors' relationship got set heavily between the visitors' energy need and the quantity of food it can harvest from flowers (Hocking 1968; Churchill and Christenensen 1970; Hainsworth and Wolf 1972a, b, c; Heinrich and Raven 1972; Stiles 1971) which influence the frequency of visits to flowers (Heinrich 1973; Heinrich 1975a, b). Because the social set up of honeybees requires a large quantity of food supply for their brood, many more times their own energy requirements are secured by repeated visits to flowers (Reddy and Reddy 1984). This can be sustained only if a flower provides sufficient rewards to attract the foragers on one hand and limit the reward on the other hand compelling the visitors to frequent other flowers of the same species (Heinrich 1975a, b), thus enabling development of an optimum strategy both by flowers and pollinators for maximum cross-pollination (Emlen 1966; Schoener 1971; Cody 1974).

11.2 Diversity of Honeybees in Asia

The Asian continent is richest in the world in the honeybee species diversity. A number of species, e.g. *Apis cerana*, *Apis dorsata*, *Apis florea* and *Apis laboriosa* are indigenous to the region. In addition to these, a number of other species of *Apis* have been identified. These are native to the region whereas *Apis mellifera* has been introduced and is being widely used for honey production (Ruttner 1988).

In the past, genus *Apis* was believed to have four species: *A. mellifera* Lin. (species native to Europe and Africa with 24 sub-species), *A. cerana* Fab. (Asian species with 4 sub-species), *A. dorsata* Fab. and *A. florea* Fab. In 1980, the largest bee species in the world, *A. laboriosa* Smith was reconfirmed from higher altitudes of Nepal and in 1987, the world's smallest bee, *Apis andreniformis* Smith having black body colour and living in Southeast Asia was reconfirmed as an independent species from *A. florea* Fab. Similarly, in 1988, a red honeybee, *Apis koschevnikovi* Enderlein discovered in Sabah, East Malaysia was another independent species from *A. cerana* Fab. Then in 1996, *Apis nigrocinta* Smith in Sulawesi Island, Indonesia and *Apis nuluensis* Lin. in same area as the habitat of *A. koschevnikovi* Enderlein were described as two new species. Hence, among these nine species, eight species are distributed in Asian countries under different agro-climatic conditions as detailed below:

1. *A. dorsata* Fab., Rock Bee Asia
2. *A. florea* Fab., Little Bee Asia
3. *A. laboriosa* Smith., Largest Bee Asia
4. *A. cerana* Fab., Asiatic Hive Bee Asia
5. *A. andreniformis* Smith., Smallest Bee Asia
6. *A. mellifera* Lin., European Bee Europe
7. *A. koschevnikovi* Enderlein., Red Bee Malaysia
8. *A. nuluensis* Lin., Malaysian Bee Malaysia
9. *A. nigrocinta* Smith., Black Bee Indonesia

Of the nine species of honeybees, *A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nuluensis* and *A. nigrocinta* all build nests containing a series of parallel combs. These species usually nest in cavities. *A. florea*, *A. andreniformis*, *A. dorsata* and *A. laboriosa* species of honeybees whose nests consist of single combs.

11.3 Foraging in *Apis cerana*

Foraging by honeybees is a social enterprise, one in which thousands of foragers in a colony cooperate to find and exploit rich patches of flowers. The natural food sources of honeybees, the flowers, differ dramatically in their availability and predictability in both biotopes of *A. cerana* and *A. mellifera*—the tropical Asian forest and that of the European one. While in Europe honeybees exploit large flower fields that are predictable and stay in the same location for several days, in the tropical forest there are no such massive concentrations of flowers, and these may appear and

disappear in a rather unpredictable way. As a result of natural selection in their respective homeland, *A. cerana* is adept in collecting sporadic nectar flowers in the mountain and forest region, but *A. mellifera* exploits large flower fields well (Chen 2001; Zeng 2009). Furthermore, collecting propolis is peculiar to *A. mellifera* (Chen 2001). Honeybee foragers have to fly several kilometres—up to more than 10 km away—to collect pollen and nectar, and therefore it is necessary for them to learn and remember not only the colour and shape of flowers, but also how to get to them (Chittka et al. 1993; Menzel et al. 1996; Vorobyev and Menzel 1999; Zhang et al. 1999; Collett et al. 2003; Pahl et al. 2011). Therefore, we expect that the ability to track food sources may differ between both species, and thus, their ability to learn about them should also be different. Besides, in tropical regions—except those with homogeneous vegetation that provides adequate nectar and pollen—*A. cerana* colonies are likely to abscond to another area in response to cessation of flowering, while *A. mellifera* would rather be starved in the hive than migrate (Gong and Zhang 2000). Honeybees must learn and adapt to new surroundings when migrating, which we hypothesize may also cause some difference in the learning and memory between the two species.

11.4 Pollinator Foraging Behaviour

11.4.1 Pollen and Nectar as Food Resources

In the mutual relationship of pollination, the interactors have an inherent conflict of interests: plants need bees as pollen vectors but bees only use flowers as a food source. Originally plants offered pollen as food; nectar, which has a water component, evolved secondarily, to provide a food resource that is less costly to the plant.

Bees use pollen as the prime protein source for raising larvae. Unlike bumblebees, honeybee larvae do not consume pollen directly throughout their entire development. During their early development, they are fed by glandular secretions of adult workers, who eat pollen both to feed the larvae (Dobson and Peng 1997; Hrassnigg and Crailsheim 1998; Babendreier et al. 2004) and to satisfy their own protein needs (Smeets and Duchateau 2003).

Chemically the pollen consists of proteins, starch, carbohydrates, lipids and pectines (Runge 1973). Pollen grains consist of three layers: the outer exine (pectines and lipids), the intine (starch and carbohydrates), and the inner protoplasm, which contains mostly proteins (Dobson 1991). The exine layer greatly resists digestion, but is perforated by pores that lead to the intine (Roulston and Cane 2000). Storing the pollen helps to ease the digestibility of the pollen grain when it comes into contact with nectar and enzymes (Dobson and Peng 1997; Roulston and Cane 2000; Human and Nicolson 2006).

The nutritional values of pollens vary among plant species and for insect species. Food preference may be based not only on the chemical composition (Dobson 1988; Kim and Smith 2000; Roulston and Cane 2000; Cook et al. 2003; Markowicz Bastos

et al. 2004), protein concentration (Rasheed and Harder 1997; Roulston et al. 2000; Roulston and Cane 2000; Saa-Otero et al. 2000; Pernal and Currie 2001) and caloric value (Colin and Jones 1980; Petanidou and Vokou 1990), but also on the digestibility of different pollens (Dobson and Peng 1997; Franchi et al. 1997; Roulston and Cane 2000).

Nectar is a liquid substance that serves both as an attraction to the bee and as a reward for the pollination service (Baker and Baker 1973). Of the several hypotheses about the origin of floral nectar, the most widely held is the 'leaky phloem' hypothesis: the phloem is also the sugar-rich solution that provides flowers with nutrients (Barrera and Nobel 2004). In addition to water and sugars, nectar contains small quantities of amino acids, lipids, antioxidants, minerals and secondary metabolites (Faegri and van der Pijl 1979; Gardener and Gillman 2001a; Gardener and Gillman 2002; Barrera and Nobel 2004). The presence and amount of trace elements in the nectars may explain why some sugar-rich nectars are not or less attractive to bees (Adler 2000; Afik et al. 2006). The exact chemical composition of nectar varies among the plant species (Faegri and van der Pijl 1979; Adler 2000), flower types and even the soil properties of a particular location (Waser and Mitchell 1990; Gardener and Gillman 2001b). For bees, nectar is important as an easily assimilable energy source (Faegri and van der Pijl 1979) and as the proper medium for supporting the digestion of pollen grains (Roulston and Cane 2000).

11.4.2 Foraging as a Function of Energetic Relationship

11.4.2.1 Foraging in Relation to Reward from Flowers

Pollinators differ in their energy requirements from low energy groups such as ants and flies to higher energy requiring endothermic group of mammals and birds (Heinrich 1983). Besides, colour, shapes and odour of flowers, energy requirement and caloric rewards offered by the flowers also determine whether or not an animal can be a dependable flower visitor (Heinrich and Raven 1972; Heinrich 1975a; Abrol 1985, 1986a, b, c, 1991a, b, c, 1992a, b, 1993a, 1995a, b, c, 1998a, c, 2005, 2007a, b). Rewarding system developed by flowers enable bees to make distinction between them and closely related species or ecotypes. This has resulted in a mutualistic development of a co-evolutionary process and have evolved co-partnership between the two (Leppik 1977). The interdependence of pollinating bees with flowers depends much upon their energy requirement and the balance sheet they share with flowers (Heinrich and Raven 1972). The pollinators with high energy requirements may not forage at the flowers, which provide low caloric rewards (Heinrich 1983).

Energetics of pollinator plant interaction has been investigated in only few instances. Many studies of foraging economics have focused on birds especially nectarivores (Stiles 1971; Wolf and Hainsworth 1971; Gill and Wolf 1975) as they are usually easy to observe and their activities are easily classifiable into discrete

categories (e.g. flight, rest and torpor). Evidently, their energy intake is rather easily quantified. Some interesting information on bumblebees and honeybees has also been generated (Heinrich 1975a, b; Waddington et al. 1981; Schaffer et al. 1979). Bumblebees and honeybees have been widely used in studying foraging energetics because of their high energy requirements. Their foraging patterns are largely determined by the relationship of energy requirements to flight to nectar reward in flowers (Heinrich 1975a, b, 1983; Wolf et al. 1972, 1975). The high energy requirements of bumblebees and honeybees result in part from their social organization and ability to thermoregulate (Heinrich 1983)

Most bee–plant interactions depend upon the energy needs of the pollinators and the energy available from plants (Heinrich and Raven 1972; Heinrich 1975a, b, 1979a, b, 1983; Abrol 1986a, 1992a). The foraging strategies of bees and allocation of time to various daily activities is probably related to their level of sociality. Honeybees, which are active throughout the year, need continuous influx of energy for thermoregulation and brood development. The solitary bees in contrast to honeybees have less energy demands due to their individual nesting habits, as they need not incubate brood or support nest mates. Solitary bees can be oligolectic/polylectic, whereas longer-lived colonies of eusocial bees are largely restricted to polylecty, which can affect their foraging efficiencies at certain floral hosts (Weislo and Cane 1996). Evidently, foraging strategies of honeybees could be entirely different from earlier studied wild solitary bees.

Abrol (2007) studied the energy used by a bee *A. cerana* and *A. mellifera* in relation to various rapeseed, mustard (*Brassica juncea*, *Brassica carinata*, *Brassica campestris* var. *toria*) and umbelliferous crops (*Foeniculum vulgare* and *Coriandrum sativum*). The energy balance was found to vary from 1 day to another and from crop to crop. In general, the foraging profitability per bee was in the order: *B. carinata* > *B. juncea* > *B. campestris* var. *toria* > *F. vulgare* > *C. sativum* for both the species. *B. carinata* appears to be competitively superior as a forage crop over *B. juncea* > *B. campestris* var. *toria* > *F. vulgare* > *C. sativum*. He further found that the flight activities of *A. cerana* commenced much earlier and ceased later than *A. mellifera*. Evidently, *A. cerana* worked for longer durations in the field as compared to *A. mellifera*, *A. cerana*, on an average spent 300.0 min in foraging, 336.78 min in nest activity and 803.21 min resting as compared to *A. mellifera* which under similar conditions spent less time in foraging (284.28 min), nest activity (222.50 min) and more time in resting (933.21 min). *A. cerana*, on an average made 10–15 foraging trips/day for nectar collection and each trip averaged between 23.0 and 25.0 min while as *A. mellifera* made 10–12 trips/day and on an average spent 26.5 min/trip.

In a similar study, Abrol (2006a) observed the foraging behaviour of four honeybee species (*A. mellifera*, *A. cerana*, *A. dorsata* and *A. florea*) in relation to energy production rates of *Prunus persica* and a simultaneously blooming weed, *Lepidagathus incurva*. Energy produced ranged from a minimum of 0.642 ± 0.01 J/flower/day (*L. incurva*) to a maximum of 1.49 ± 0.14 J/day (*P. persica*). The weed having higher nectar sugar concentration and high flowering density attracted more number of bees as compared with peach. The foraging rates of bees were much higher on the weed and they could harvest more energy per unit time from the weed flowers. The results

indicate that food acquisition efficiency and quality of food determines the foraging decision of bee. Bees are generally attracted to flowers that are available in large numbers and are more rewarding in terms of energy (Abrol 1993a). Nagamitsu and Inoue (1999) found that pollen collection from seven plant taxa differed between *A. cerana* and *A. mellifera*; *A. cerana* preferred tall trees, while *A. mellifera* favoured short herbs.

Similarly, the influence of mustard, radish, and clover in detracting honeybee pollination of apples caused negative impact on apple pollination (Kakar 2000). Verma (1995) also observed in his study that when alternate sources were available *A. mellifera* did not prefer to forage on the apple flowers. These investigations revealed that the bees prefer to forage on more attractive plants and neglect the others when they flower at the same time and location affecting the pollination requirements of the less attractive plant species to a greater extent.

The pollinators with high energy requirements may not forage at the flowers which provide insufficient caloric reward (Heinrich 1983). Smaller flowers with low caloric rewards are unattractive to larger hovering animals which cannot meet their energetic requirements through them. In general, size of the flower and caloric rewards in relation to size of visitor and energy requirements seem to be determinants for resource partitioning among the various bees and thus permitting co-existence under similar ecological conditions. The population of certain species of pollinating bees was found to be the function of their body size as well as the size of the flowers, since the feeding pattern of many animals suffer as a function of their triplicate structure (Heinrich 1976, 1979a, b; Harder 1986, 1985).

11.4.3 Foraging Preference of Bees in Relation to Nectar Sugar Components

Sihag and Kapil (1983) found that *A. dorsata* with higher energy requirements visited sucrose dominated flowers more frequently than did *A. florea* which mostly relied on glucose dominated ones. Wyke (1952) reported that honeybees preferred equiproportioned sugars. Waller (1972) and Abrol (1985) on the contrary stated that bees prefer nectar with one dominant sugar than the equiproportioned sugars. Abrol and Kapil (1991) studied the foraging strategies of honeybees and solitary bees in relation to nectar sugar components of 13 crops plants and found that honeybee *A. dorsata* and solitary bees *Megachile lanata* Lepel, *Megachile cephalotes* Smith and *Xylocopa fenestrata* F. visited sucrose dominated nectars more frequently than did *A. florea* F. and *Pithitis smargdula* F. which heavily relied on glucose dominant nectars. Preference of sucrose or glucose dominant nectar indicates that total caloric reward is an important factor determining the attractiveness of foraging population on a particular crop (Abrol 1989a, b; Abrol et al. 1989; Abrol and Kapil 1991). Abrol (1991) reported that *A. dorsata* bees were generally most active between 1100 and 1400 hours when temperature ranged between 24.5 and 34.5 °C, relative humidity (RH) between 22.5 and 43 %, light intensity between 5,100 and 5,500 lx and solar

radiation between 60 and 85 mW/cm², soil temperature between 22.5 and 32 °C, nectar sugar concentration between 32.00 and 45.00 % and wind velocity between 2.9 and 4.2 km/h. Increase in temperature above this range resulted in decline of bee activity. Of the seven factors studied, the direct effect of light intensity, solar radiations and soil temperature were positive and other factors were negative.

11.4.4 Nectar Productivity and Bee Activity

One source of variation in foraging cues between genotypes is the nectar which may provide variety of stimuli. Nectar is the potential energy reward provided by the flowers to their visitors. It has been found to be very significant parameter that decisively shapes the behaviour and physiology of pollinators in relation to their energy demands. (Heinrich 1975a, b; Abrol 1986a, 1990, 1995a, 2007a, b, 2009, 2010a, b). Abrol (1990) studied the energetics of nectar production in 54 cultivars of apple in relation to foraging behaviour of *A. cerana* and *A. mellifera* and found that cultivars varied greatly in pattern of nectar production characteristics and pollinator attractiveness. Cultivars providing higher amount of sugar, nectar concentration and energy were highly attractive to bees which was reflected in their population dynamics. In a similar study, Abrol (1992c) studied foraging preferences of honeybee *A. cerana* and *A. mellifera* in relation to nectar productivity of 13 cultivars of strawberry (*Fragaria* sp.). Foraging of both *A. cerana* and *A. mellifera* correlated with energy yields. The results suggest that cultivars with higher caloric rewards had a competitive edge over others in attracting foraging populations of both the species. Similar results were obtained in relation to the foraging preferences of honeybees *A. mellifera* and *A. cerana* on almond cultivars (Abrol 1995a). Abrol (2010a, b) found that four honeybee species *A. dorsata*, *A. mellifera*, *A. cerana* and *A. florea* foraging on peach and a weed *L. incurva* had a differential attractiveness related to flower density and availability of floral rewards

11.4.5 Flower Odour as the Basis for Searching Food

Olfactory signals have been thought to be the most important signals for the bees' recognition of food sources while foraging (Kriston 1973; Menzel et al. 1993). However, scientists have argued the prevalence of colour versus scent. The priority of colour over scent has been found in halictid bees (Roy and Raguso 1997) and in one butterfly species (Ômura and Honda 2005).

The chemical signals may act directly as long or short distance attractants, or may function as indirect cues: young bees remember the smell of the food they ate inside the hives and search for it during their first foraging trip. Bees are able to differentiate a large number of olfactory signals and learn to predict foods which offer rewards and which do not (Menzel et al. 1993; Laska et al. 1999; Gumbert and Kunze 2001). Since

floral odours are blends containing tens to hundreds of components which vary in quality or quantity over time and in space, the generalization process is fundamental for bees' survival (Sandoz et al. 2001). From the floral extracts, comprising multiple components, bees restrict the number of scent components they use in their searching (Pham-Delègue et al. 1997; Laloi et al. 2000). It is considered possible that bees may avoid plants treated with pesticides due to the repellent odours of the compounds (Shires et al. 1984).

11.4.6 *Flower Constancy*

Of all flower-visiting insects, bees exhibit a high level of flower constancy; in fact, it is their behavioural specialty. They must gather food not only to feed themselves but also their brood. Foraging bees often show a kind of flower constancy favouring some and bypassing others that might offer rewards (Free 1970a, b; Gegear and Thomson 2004). It has been claimed that bees have no innate flower colour preference (Waser and Price 1983; Gumbert and Kunze 2001) but develop them over time, testing various flower types until they find one that offers a reward (Pohtio and Teräs 1995). It is suggested that flower constancy may have evolved to save energy and/or time for the foragers (Free 1970a, b; Dukas 1995; Gegear and Thomson 2004). The level of flower constancy varies among bee species even belonging to the same genera (Free 1970a, b). Complex flowers require more handling skills than do simple flowers. A bee that is morphologically suited to the flower shape is able to learn the easiest way to obtain a reward (Laverty 1994; Gegear and Laverty 1998; White et al. 2001).

Both memory and the learning capacity of insects are usually under-estimated. Studies in honeybees (under both natural and laboratory conditions) demonstrate that learning is fast and comprises various levels of cognitive processing, such as generalization, categorization, concept formation, configuration and context-dependency (Menzel and Giurfa 2001; Gegear and Thomson 2004).

The honeybee's memory is rich, highly dynamic and long-lasting (Menzel 1999). Despite that, it is probably not feasible for them to store the locations of several hundred individual flowers (Goulson 2000). Although they may restrict the number of flower choices due to their limited memory patterns (Dukas 1995; Menzel 2001a, b), they also create new scents to improve their foraging. When collecting nectar or pollen, they deposit short-lived repellent odours on the flower corolla to ease demands on memory (Cameron 1981; Free and Williams 1983; Goulson et al. 2000; Goulson et al. 2001; Stout and Goulson 2001). These marks can be used intra- or interspecifically to avoid visiting empty flowers (Goulson et al. 2000; Goulson et al. 2001; Gawleta et al. 2005). However, bees do not rely entirely on those pheromone scent marks. They sometimes encounter situations when it could be profitable to ignore the scent marks and probe the newly visited flowers (Saleh et al. 2006).

Floral constancy is one of the striking features of honeybees where in a single trip, a honeybee almost invariably collects reward from just one type of flower (Free 1970a, b). Flower constancy is important for bees because they learn to extract nectar

and pollen while visiting the same flower type (Grant 1950). The constancy of bees can be estimated by examination of pollen types on the body after foraging. For example, 99 % of the pollen grains in pollen pellets carried by *A. mellifera* have been shown to be from the same species of plant (Stimec et al. 1997). Raj et al. (1993) found that *A. mellifera* and *A. cerana* foraging on rapeseed *Brassica campestris* exhibited pollen consistency. Pollen loads of 66.78 % collected by *A. mellifera* and 65.45 % collected by *A. cerana* were found to be from mustard crop. Priti and Sihag (1997) also observed similar phenomenon in case of *A. mellifera* and *A. cerana* visiting cauliflower (*Brassica oleracea* L. var. *botrytis* cv. Hazipur Local) blossoms at Hisar, India.

Chaudhary (1978) reported that *A. cerana indica* showed higher floral fidelity, as compared to other foragers, i.e. *A. florea*, *A. dorsata* and *A. mellifera* foraging on alfalfa (*Medicago sativa*). Later, Dhaliwal and Atwal (1986b) and Naim and Bisht (1989) also confirmed strong floral fidelity in *A. cerana* where foragers collected one kind of pollen in a single trip and continued to collect it throughout the day.

Honeybees' floral constancy makes them well-suited for pollination purposes. At any one time, a hive of bees may be collecting pollen from dozens of different plant species. Floral constancy and local foraging has an adaptive advantage leading to sympatric and allotropic speciation in orchids, which are pollinated only by male euglossine bee (Dressler 1968a, b; Dodson 1975). Nataraj et al. (2000) also reported flower constancy in *A. cerana* under Tamil Nadu conditions, whereas Thiyagesan et al. (2001a, b) reported on the risk sensitive and central foraging in honeybee *A. cerana*. Devkota and Thapa (2005) found that the pollen loads of *A. cerana* bees in March and June were mostly from coconut (90 %), graminiae (6 %) and jackfruit (4 %). In *A. mellifera* (67 %) pollen loads were from coconut, (25 %) from graminiae and (10 %) from Rubiaceae, thereby indicating floral constancy in collection of pollen.

11.4.7 Species-Specific Variations in Foraging Activity

Abrol et al. (2005) studied the foraging activity of honeybee *A. cerana indica* and *A. dorsata* on peach flowers in relation to weather conditions and found that initiations of flight was a dual threshold of temperature and light intensity and cessation controlled by reduction in light intensity for both the bee species though the temperature was still over the levels required for initiation of the activity. Foraging population correlated significantly and positively with ambient temperature, light, solar radiations, nectar sugar concentration and negatively with RH. Only one factor namely light intensity directly influenced the flight activity of *A. cerana indica* while the flight of *A. dorsata* was influenced both by temperature and light intensity. This demonstrates that different yet closely related bee species differ in their responses to environmental conditions. The differences in response of the bees are species specific and indicative of their physiological adaptations. For instance, Sihag and Abrol (1986) found that for *A. florea* RH and solar radiations were the important factors

Table 11.1 Flight speeds and wing-loading of honeybees

Species	Flight speed (m/s)	Wing-loading (N/m)	Reference
<i>A. florea</i>	4.81	7.28	Dyer and Seeley (1987)
<i>A. cerana</i>	7.17	7.84	Dyer and Seeley (1987)
<i>A. dorsata</i>	8.12	13.02	Dyer and Seeley (1987)
<i>A. mellifera</i>	12.61	14.21	Seeley (1986)

influencing flight activity. Burill and Dietz (1981) found solar radiations important for *A. mellifera*. Nunez (1977) reported that in *A. mellifera* the morning activity was related to nectar flow, whereas the afternoon activity was correlated with the photo period. Bailey et al. (1982) reported on the role of humidity whereas Lerer et al. (1982) emphasised solar energy in the pollination activity of *Megachile rotundata*.

Abrol (1992a) studied the foraging strategies of six bee species in relation to 25 crops. *A. dorsata* with its larger size and higher energetic demands preferred *Cajanus cajan*, *Parkinsonia aculeata*, *Pongamia glabra* and *Luffa cylindrica* than flowers of *F. vulgare*, *C. sativum*, *Daucus carota*, *Allium cepa*, *Trigonella foen-graceum* and *Mangifera indica*. The latter group of flowers was visited by *A. florea* through the day. The latter bee species with its small size and body weight are psychologically better adapted to extract maximum reward from these flowers. In case of *Brassica* crops, *A. dorsata* visited flowers during early hours of the day at peak periods of nectar production. But 1100 hours onwards foraging population shifted to *Trifolium alexandrium* due to reduction in quantity of reward available from *M. sativa* and *Brassica* crops. *A. florea* which commenced activity between 1000 and 1100 hours dominated throughout the day. In case of sunflower all type of pollinators continued foraging throughout the day. Though the caloric reward per flower is low yet each bee species was able to maintain energy balance, since flower heads with platform provide no barrier for landing of the foragers, the energetic cost is reduced due to temporary suspension of hovering flight and large number of flowers can be visit in rapid succession.

11.4.8 Speed of Flight

In honeybees, the speed of flight has been found to vary from 20.9 to 25.7 km/h (average 24 km/h) for loaded bees and from 10.9 to 29.0 km/h (average 20 km/h) for empty bees. This is perhaps due to the fact that bee, on outward flight does not make a beeline in all the cases for the source of supply. Bees do not like to work in a wind blowing over 24 km/h. The speed of flight of honeybees is given in Table 11.1.

11.4.9 Thermoregulation During Foraging

The honeybee's ability to thrive in a vast range of environments, from temperate regions to humid tropical and hot desert habitats, reflects powerful temperature

control not only by whole colonies inside their nests, but also by individual foragers out amidst the flowers. These bees must maintain their body temperature below the lethal upper limit of 45–50 °C, and while flying, must keep their thorax temperature above 27 °C, the lower limit for steady flight. The foraging bees manage to fulfil these requirements across the range of ambient temperatures from about 5 to 45 °C thus achieving considerable freedom from temperature limitations when collecting resources for the colony. The stop-and-go foraging is expensive in time and energy spent on warm-up, but can still be profitable when working on rich flowers, especially since once a bee reaches the flowers her behaviour will likely consist of intermittent flight between flowers, thus automatically affording her opportunity for warm-up when perched on two flowers. Honeybees do possess an effective cooling system for flight at high temperatures. The primary mechanism for cooling the thorax appears to be to keep the head cool by regurgitating fluid from the honey stomach and holding the extruded nectar triplet between the tongue and mandibles.

Dyer and Seeley (1987) found that thoracic flight temperature in foragers of the three Asian honeybee species (genus *Apis*), which, together with the European species *A. mellifera*, span a five-fold range in body mass from the smallest species to the largest. Over a 15 °C range in ambient temperature, they found that thoracic flight temperature in each species is strongly dependent upon ambient temperature. However, the temperature gradients (thoracic flight temperature–ambient temperature) at a given temperature, do not appear to increase with body size in the four species. The smallest species, *A. florea*, shows the smallest thoracic flight temperature–ambient temperature, but the intermediate-sized *A. cerana* and *A. mellifera* both show a consistently higher thoracic flight temperature–ambient temperature than the largest species, *A. dorsata*. Furthermore, compared on a mass-specific basis, *A. dorsata* and *A. florea* are more similar to each other than either is to the other two species. This physiological dichotomy among the four species parallels a dichotomy in nesting behaviour and colony demography (Singh et al. 2007).

11.5 Floral Rewards

11.5.1 Nectar

Nectar is the primary floral reward and food source offered by plants to attract pollinators. Nectar variation can be ecologically important because pollinators often exhibit a preference for certain types of nectars over others (Van Riper 1958; Hainsworth and Wolf 1976; Stiles 1976; Pyke and Waser 1981; Tamm and Gass 1986; Alm et al. 1990; Erhardt and Rusterholz 1998), which can affect the type of plants that are pollinated. Variation in nectar traits can also be important evolutionarily. Phenotypic variation in nectar traits can have both a genetic and environmental component. If variation of a nectar trait has a genetic component, the trait can be subjected to selective pressure if it is also heritable and affects plant fitness. If selective pressure is placed on a nectar trait through pollinator preferences for certain nectar types over

others, it could result in ethological isolation, which has the potential to promote species divergence or maintenance during secondary contact (Grant 1994).

11.5.2 Nectar Foraging

Strength of a bee colony is important for surplus honey gathering. Sharma and Sharma (1950) found that *A. cerana indica* colonies of 18,000 bees were unable to give any surplus while colonies of 12,000 bees failed even to supply their own needs; colonies of 20,000 or more gave surplus. Nectar gathering capacity per 100 bees increased steadily as the number of bees in the colonies increased. Amount of syrup collected by the foragers of this species was measured for sugar concentration of 20, 30, 40, 50 and 60 % of their body weight as compared to 82 % in *A. cerana indica* and *A. mellifera* (Singh 1971). Average nectar load of *A. cerana indica* and *A. mellifera* foraging on *Plectranthus rugosus* was 18 and 27 μ l, respectively (Gupta et al. 1984). Diwan and Rao (1969) observed that *A. cerana indica* foragers collected water prior to visiting the white sugar-rings of dehydrated nectar secreted by the exposed nectaries of *Synadenium grantii* Hook.

Mattu and Verma (1985) reported that *A. cerana indica* in Simla hills of north-western Himalayas showed peak of nectar collection after peak of pollen collection daily. Over the whole year there were more nectar collectors than the pollen collectors, pollen + nectar collectors or water collectors. But there were considerable variations in the percentage of nectar collectors as compared to the pollen, pollen + nectar and water collectors during different seasons. Dhaliwal and Atwal (1986b) observed the effect of age of crop, soil moisture and phosphate fertilizers on the nectar production in *Brassica* crop and their effect on the foraging activity of bees. Kapil and Kumar (1975) reported nectar robbing by *A. dorsata* on *B. juncea*.

Sihag (1983) observed *A. dorsata* and *A. florea* bees foraging on the juice of ruptured/damaged grapes (*Vitis vinifera* L.) during dearth period. The grape juice contained 14–20 % dissolved sugars and was comparable with nectars of cruciferous crops in having glucose dominated sugars. Around 10,000–45,000 bees/ha of *A. dorsata* and 20,000–60,000 bees/ha of *A. florea* were attracted on damaged grapes.

11.5.3 Pollen Foraging

Foraging pattern showed inverse relationship of pollen gathering activity with nectar gathering and non-foraging activity (Reddy 1980a). Pollen gathering activity of *A. cerana indica* in Delhi was reported by Bisht and Pant (1968). The bees collected pollen throughout the year and maximum was from January to March. But in Shimla hills of north Himalayas, these bees collected pollen and nectar throughout the year (Mattu and Verma 1985). Foraging activity was greater in summer and autumn than in winter and in monsoon periods. Time of greater flight activity varied from season to season. The correlations between pollen foraging activity and temperature and RH

were also given. Similar observations were reported by Singh (1981) in Saharanpur and also found that foraging hours varied with season of the year. Verma (1983) found that peak pollen collected by *A. cerana indica* in Jeolikote was between 0800 and 1100 hours in February–March. Proportions of bees in a colony that foraged for pollen, the pollen stores in hive and amount of brood reared followed a similar pattern (Reddy 1980b). Thakur et al. (1982) on comparative foraging behaviour reported that *A. cerana indica* on mustard had considerable activity in the morning and *A. mellifera* picked up by 1000–1030 hours, and remained active till late in the evening. At Pune, *A. cerana* and *A. florea* commenced foraging activity on onion between 0900–0930 hours and 0815–0840 hours respectively, and continued till 1830 hours (Rao and Lazar 1980). The peak foraging of *A. cerana* was observed in the afternoon between 1500 and 1600 hours. *A. cerana indica* foragers started foraging on mustard under mid-hill conditions between 0900 and 1000 hours and stopped foraging beyond 1500 hours (Bhalla et al. 1983a; Mattu et al. 1994). But its activity on stone fruits continued up to 1600 hours (Bhalla et al. 1983b). Chaudhary (1978) analysed 5,200 pollen loads and only 56 contained pollen from more than one plant species which showed high floral fidelity of *A. indica*. Similar observations were reported by Chaturvedi (1973, 1977), Sharma (1970a, b), and Jhajj and Goyal (1979b), Dhaliwal and Atwal (1986a, b) reported that the foragers of *A. florea*, *A. dorsata*, and *A. mellifera* foraging on alfalfa (*M. sativa* L.) carried 60.0, 60.0 and 66.6 % pure pollen loads. Jhajj and Goyal (1979c, 1986) also reported that in *A. cerana indica* and *A. mellifera*, the flower constancy decreased with time; after 5 days only 20 % of the original *A. cerana indica* and 28 % of *A. mellifera* remained flower constant. A change from pollen to nectar foraging was more common than vice-versa. In caged pollination experiments, Mohan (1973) found that bees did not pollinate sunhemp flowers and collected pollen without touching the stigma. Dhaliwal and Atwal (1986a) also observed this type of behaviour in *A. mellifera*, *A. dorsata* and *A. florea* foraging on alfalfa *M. sativa*. The Indian bee *A. cerana indica* collected an average pollen load of 0.019 g from *B. juncea*, *Pyrus malus* and *Zea mays* (Punjabi et al. 1969). Little pollen was collected from maize, average pollen load from mustard was 8 mg (Naim and Bisht 1979). Dhaliwal (1970) compared the pollen collection by *A. mellifera* reared in *A. cerana indica* combs or in *A. mellifera* combs. The comb cell size was found to considerably affect the pollen carrying capacities of bee foragers. Jhajj and Goyal (1979a) have also reported reversal of pollen foraging to hive guarding by carbon dioxide anaesthesia.

11.6 Foraging Speed and Foraging Rate

Atwal et al. (1970) reported that *A. cerana indica*, *A. dorsata* and *A. florea* visited 12.7, 16.6 and 17.2 flowers of Sarson per minute. But, *A. cerana indica* was faster worker than the other two species on *Eranthemum*, radish, *Eucalyptus* and berseem. In general, *A. florea* was the slowest forager, average number of *B. juncea* flowers visited by *A. dorsata* were 12.3/min. Under mid-hill conditions, visiting mustard blooms, *A. cerana indica* foraged 10 flowers/min (Bhalla et al. 1983a; Raj and

Rana 1993, 1994) but on stone fruit flowers the rates were 6.9 on peach and 4.9 on almond (Bhalla et al. 1983b). Dhaliwal and Atwal (1980) observed that on alfalfa (*M. sativa*) foraging behaviour of honeybees was related with their body weights. But foraging rates were in the order of *A. mellifera* < *A. florea* < *A. dorsata*. On onion blooms, *A. cerana indica* was faster flier between the umbels than *A. florea* visiting 1.93 and 1.34 umbels/min, respectively (Rao and Lazer 1980). But on an umbel, *A. florea* showed better foraging rate than *A. cerana Indica* visiting 6.53 and 5.93 flowers/min, respectively. Dhaliwal and Atwal (1986a) compared the tripping efficiency of different bees visiting alfalfa (*M. sativa*), where order of efficiency was *A. dorsata* > *A. mellifera* > *A. florea*. Kapil and Kumar (1975) reported the temporal change in foraging rate of *A. dorsata* which was 9.23 flowers/min in the morning but 16 flowers/min in the evening. *A. cerana Indica* was found to be slower forager than *A. mellifera* on *P. rugosus*; these species foraged 17.5 and 25.8 flowers/min in the morning and 25.00 and 33.6 flowers/min in the evening, respectively (Gupta et al. 1984; Shah and Shah 1989). Amount and nectar concentration were responsible for morning and evening differences in the rate of foraging. Higher number of flowers of cauliflower were visited per minute by *A. dorsata* followed by *A. mellifera*, *A. cerana*, *Eristalis* spp., *Ceratina* spp., *Halictus* sp. and *Lasioglossum* sp. (Kakar 1981, 1983; Selvakumar et al. 2006). Kapil and Kumar (1975) found that *A. dorsata* on an average visited 12.30 and 10.79 *B. juncea* flowers per minute. They found that *A. dorsata* on an average visited less number of flowers of *B. juncea* during morning between 0930 and 1100 hours when the temperature was low and the number visited increased with the advance of day and their maximum visits occurred between 1430 and 1545 hours when the temperature was relatively higher.

Abrol (b) studied the foraging rate of *A. mellifera* and *A. cerana* on kiwifruit (*Actinidia deliciosa*) flowers and found that time spent on a flower averaged 33.0 s for *A. mellifera* and 38.0 s for *A. cerana*, and no. of flowers visited/min were 1.8 and 1.6, respectively.

Devkota and Thapa (2005) studied the foraging behaviour of *A. cerana* F. and *A. mellifera* L. in broccoli blooms under caged and open conditions in Chitwan, Nepal and found that both the bee species preferred open plot for foraging and *A. cerana* F. foraged significantly higher number of broccoli flowers (an average of 11.39 and 12.11 flowers/min) as compared to *A. mellifera* L. (an average of 9.03 and 10.89 flowers/min) under caged and open conditions, respectively. Chandel et al. (2004) reported that *A. dorsata* and *A. cerana* on an average visited 7.5 and 5.4 flower/umbel/visit in case of onion flowers.

11.7 The Sub-lethal Effects of Pesticides on the Behaviour of Bees

The effects of pesticides on non-target organisms have been studied extensively. It is obligatory for chemical companies to provide mortality data for their products for all larger organism groups. But, despite research data indicating the severe mortality

rate on insects, less attention has been paid to the sub-lethal effects. Application of insecticides is often allowed during the flowering period of a given crop. However, even when insecticides are not sprayed on flowers, the residues of the compounds still contaminate nectar and pollen in sub-lethal doses via both active and passive transport (Thompson 2001). Many insecticides have been described as safe to bees because they do not kill them, although sub-lethal doses may result in a decrease in their foraging and navigational abilities (Gels et al. 2002). Under certain circumstances, the sub-lethal effects may cause more harm than lethal doses since they affect the survival of the brood and colony. In the colonies of social insects the division of labour plays an important role. Each worker has specific, often age-dependent tasks. Treatment of honeybees with juvenile hormone analogues, (synthetic hormone-like compounds used as insecticides), results in a decreasing ability of young emerging bees to feed larvae, due to the early degeneration of the hypopharyngeal glands and precocious foraging ability (Tasei 2001). Changes in the division of labour of honeybees—decreased house cleaning abilities, delayed onset and duration of foraging and handling of nectar—have also been recorded (reviewed by Thompson 2003). Organophosphate insecticides may decrease the longevity of honeybees (Johansen and Mayer 1990). Juvenile hormone analogues also affected the overwintering of colonies (Thompson et al. 2005).

Foraging depends on the bee's ability to discriminate odours, to learn, to communicate, and to orientate to its environment; altering these systems may result in a decrease in foraging. The bees' orientation and communication ability have been found to be affected by sub-lethal doses of organophosphorus (Schricker and Stephen 1970), synthetic pyrethroids (Cox and Wilson 1984; Vandame et al. 1995) and at least one neonicotinoid (Bortolotti et al. 2003). Pyrethroids and neonicotinoids have also been shown to affect both foraging activity (Thompson 2003) and learning capacities (Decourtye et al. 1999, 2003; Guez et al. 2001; Ramirez-Romero et al. 2005). Pyrethroids may also affect thermoregulation (Jagers op Akkerhuis et al. 1999; Belzunces et al. 2001); in cooler climates, that can lead to decreased flying ability. The decrease in foraging and in returning foragers reduces the brood production (Thompson 2003), and weakens a colony's potential for surviving the winter.

The reduction of the brood may have more damaging consequences for honeybees than simply the moderate loss of foragers (Haynes 1988). Aside from brood mortality there can be changes in larval development (both prolonged development time and malformations may occur) due to the contamination of the food by pesticides (Tasei 2001). Some organophosphates have affected the queen's status or have interfered with a colony's ability to requeen itself (Stoner et al. 1985; Thompson et al. 2005). In solitary bees, pyrethroids have been found to affect the queen's fecundity (Tasei et al. 1988). Neonicotinoids (Tasei et al. 2000) and organophosphates (Johansen and Mayer 1990) have decreased the bumblebees' brood production.

In addition to ignoring the sub-lethal effects of insecticides, there exists the problem of extrapolating data from honeybees to bumblebees. Pesticide risk assessments for honeybees are based on hazard ratios which rely on application rates and toxicity data that are unlikely to be appropriate for bumblebees. Bumblebees are active at

different times and on different crop species and, therefore, are likely to have different exposure profiles. Unlike honeybees, deaths of bumblebees due to pesticides are unlikely to be reported, since the bees are not kept domestically and die in small numbers (Thompson and Hunt 1999).

11.8 Foraging in Relation to Weather Factors

The physical environment influences the flower visiting, foraging behaviour and effectiveness of pollination in complex ways. Foraging activities of pollinating insects are under the key control of different environmental variables.

11.8.1 Commencement and Cessation of Foraging Activity

Each bee species was found to have its specific ecological threshold below which activity does not occur (Osgood 1974; Szabo and Smith 1972; Reddy 1979; Lerer et al. 1982; Burill and Dietz 1982). The time of commencement of bee activity varies from one day to another which depends upon attainment of minimum threshold conditions for their foraging activity. Abrol (2006b) studied the foraging behaviour of four honeybee species *A. dorsata* F., *A. mellifera* L., *A. cerana* F. and *A. florea* F. on litchi flowers (*Litchi chinensis* Sonn.) in relation to weather factors and found that 16 °C temperature, 74 % RH, 600 lx light intensity and 10 mW/cm² solar radiation for *A. dorsata*; 16 °C temperature, 75 % RH, 800 lx light intensity, 10 mW/cm² solar radiation for *A. mellifera*; 15.5 °C temperature, 76 % RH, 600 lx light intensity, 9 mW/cm² solar radiation for *A. cerana* and 18.5 °C temperature, 64 % RH, 1700 lx light intensity and 20 mW/cm² solar radiation for *A. florea* appeared to be minimum threshold condition for initiation of flight activity. Cessation of activities in all the honeybee species was governed mainly by decline in values of light intensity and solar radiation irrespective of other factors. Earlier, Kapil and Kumar (1975) also reported 15–18 °C as the minimum threshold temperature for commencement of field activities in honeybees. In a similar study, Osgood (1974) reported that ambient temperature is the predominant factor governing the morning flight of *M. rotundata* while cessation of activity is governed by light intensity. Abrol and Kapil (1986) made similar observations on *Megachile* species. In honeybees, *A. mellifera* and *A. cerana*, air temperature acted as a stimulus for the initiation of flight activities while cessation was controlled by decline in values of light intensity and solar radiation (Abrol 1998b, c). Osgood (1974) found that ambient temperature was the predominant factor in initiating morning flight activity of *M. rotundata* while cessation was governed by decline in light intensity.

Abrol (2006b) studied the foraging behaviour of honeybee *A. florea* F. on carrot (*D. carota*) flowers and found that flight activities of *A. florea* commenced between 0726 and 0744 hours in the morning when temperature ranged between 19.4 and 25 °C, RH between 51.0 and 75.0 %, light intensity between 1,300 and 4,700 lx and

solar radiation between 38.0 and 68.0 mW/cm². Cessation of activities was governed mainly by decline in values of light intensity (500–1300 lx) and solar radiation (10–43 mW/cm²) which were appreciably low at cessation than at the commencement. He further found that temperature, light intensity and solar radiation were the three most important factors which exerted pronounced and positive direct effect on flight activities of *A. florea* while RH had negative direct effect. Direct effect of nectar sugar concentration was positive and negligible.

Sihag (1984) studied the limiting effect of light intensity, ambient temperature and humidity on the commencement and cessation of flight activity of *A. dorsata* and *A. florea*. *A. dorsata* maintained foraging activity when illumination was as low as 0.5–1.0 lx during nights of May–June, but foraging activity was limited if the temperature was below 16.5 °C or above 38 °C and if humidity was below 32 % or there were rains. On the other hand, in *A. florea*, illumination below 200 lx, temperature below 18.5 °C or above 40 °C and humidity below 25 % or if there were rains, caused the cessation of flight activity. The highest limits of light intensity for *A. florea* were 6,500 and 7,000 lx, respectively. But before the approaching of these limits, temperature had also crossed the upper limiting values. However, once commenced, the flight activity in *A. florea* was maintained by the direct effect of solar radiation and RH. Temperature, RH and nectar sugar concentration had no direct effect on the flight activity (Sihag and Abrol 1986). Dhaliwal and Atwal (1986a) reported 33 °C as the optimum field temperature for peak activity of *A. mellifera* on alfalfa (*M. sativa*). Severe robbing during dearth period was also reported between *A. cerana indica* and *A. mellifera* (Atwal and Dhaliwal 1970; Atwal and Sharma 1971; Adlakha and Sharma 1974, 1975).

Abrol (b) studied the foraging behaviour of honeybees *A. mellifera* and *A. cerana* on kiwifruit (*A. deliciosa*) and found that peak foraging was recorded between 1100 and 1400 hours for *mellifera* (temperature 24.0–28.5 °C, RH 63–73 %) and between 1000 hours and noon for *A. cerana* (temperature 21.5–26 °C, RH 72–77 %).

A. laboriosa bees live in the Himalayas under harsh conditions at altitudes of 1,200–3,600 m where a temperature range of 4.5–23.4 °C is encountered. The lower range of temperatures recorded in *A. laboriosa* territory does not occur in *A. dorsata* territory (6 and 27 °C), and the upper temperature range recorded in *A. dorsata* territory does not occur in *A. laboriosa* territory. However, a large temperature range is the same for the active life of both species mentioned. According to Dyer and Seeley (1987) *A. dorsata* workers are not able to fly at temperatures lower than 17 °C. Woyke et al. (2003a) in a similar study found that closely related *A. dorsata* workers started foraging at a temperature of 18 °C, and *A. laboriosa* at a lower temperature of 10 °C. The increase in temperature to 12 °C resulted in a ten-fold increase in the number of foragers leaving the nests of both species, and their flight activity reaction to temperature changes was similar. High correlation between the temperature and the number of foragers was found in both species, at temperatures below 16 °C. The environmental conditions in which the bees were living for longer period influence their behaviour more than the phylogenetic relationship.

According to Lundie (1925) the lowest temperature at which the flights of *A. mellifera ligustica* began was 10 °C, but the flight commenced normally between 12 and

14 °C in April. Thus, it can be concluded, that in a similar temperature range and weather condition, the starting temperature for foraging and response of flight activity to temperature changes are similar among *A. laboriosa*, *A. mellifera mellifera* and *A. mellifera ligustica*. The lower number of foragers flying after noon than before noon, at the same temperature, may be in a major part due to the direction of meteorological changes. Woyke et al. (2000) found that during an eclipse of the sun, a lower number of *A. mellifera* workers left the hive before the maximal eclipse when the light intensity decreased, than after the eclipse, when light intensity increased. Thus, although strong correlation between temperature and flight activity was found, some other characters, like the direction of light intensity changes and others, influence the flight activity. However, both species *A. laboriosa* and *A. mellifera* reacted similarly. Szabo (1980) found a significant correlation ($r = 0.78$) between temperature (range 14–24 °C) and flight activity of *A. mellifera*. However, at higher temperatures, no correlation between those two factors was found, (Domagala Lipińska 1962; Lee et al. 1987), and at the highest temperatures, above 30 °C, flight activity decreased with temperature increase (Gary 1967; Woyke 1992). These differences are explained by Woyke et al. (2003b), according to which the correlation between air temperature and flight activity is strong at lower temperatures, but is weaker or does not exist at higher temperatures. Other bees like *A. cerana* and *A. mellifera* are already foraging at those temperatures (< 15 °C). This indicates that territories in which the morning temperatures are lower than 18 °C are the expansion ones in which *A. dorsata* is not able to take advantage of all food available. When the early morning temperatures are lower, *A. dorsata* workers start foraging at higher temperatures. This suggests that more heat is required to warm the outer worker layer of nest curtain. *A. laboriosa* workers start foraging at 10 °C, which is at temperature 6–8 °C lower than the closely related *A. dorsata* workers do. Thus, the Himalayan bee can collect food in lower temperatures. The start of foraging, in relation to ambient temperature, and the reaction of flight activity to temperature and other meteorological changes during the day, were similar in species, *A. laboriosa* and *A. mellifera*. High correlation between the temperature and the number of foragers was found in both species, for morning hours at temperatures below 16 °C. Hence, the start of foraging of two closely related honeybees *A. dorsata* and *A. laboriosa* living in territories characteristic of different temperature range occurs (within the same temperature range), at different temperatures. Whereas, both the start of foraging and the response of flight activity to temperature changes is similar in two phylogenetically distant honeybee species, *A. laboriosa* and *A. mellifera* living in territories of similar temperature range. This shows that the environmental conditions in which the bees were living for longer period influence their behaviour more than the phylogenetic relationship.

Abrol (2006b) studied the foraging ecology of the dwarf honeybee *A. florea* visiting carrot flowers (*D. carota*) in relation to five environmental variables and found that the direct effect of temperature was high and positive followed by light intensity and solar radiation while the direct effect of RH was high and negative. The direct effect of nectar sugar concentration was negative and negligible.

11.8.2 Diurnal Trends in Foraging Activity in Relation to Environmental Factors

Foraging activity occurs only when ecological conditions within which foraging occurs are attained. Szabo and Smith (1972) reported that greatest foraging activity of *M. rotundata* occurred at 30 °C in bright sunshine and declined at higher temperature in Hungary. Kapil and Brar (1971) recorded peak activity of *A. florea* on toria between 21 and 25 °C temperature and 50–57 % RH during November. Similar results were obtained by Cirudarescu (1971) who found that the number of insect visitors on lucerne varied directly with temperature and inversely with RH. Benedek and Prener (1972) found that air temperature significantly affected the foraging activities of honeybees. They further reported that flower visiting rate increased with increasing air temperature. Nunez (1977) reported that morning activity of *A. mellifera* was related to nectar flow, while the afternoon, with the photoperiod. (Cirudarescu 1971; Szabo and Smith 1972; Nunez 1977; Corbet 1978a, b; Bailey et al. 1982; Burill and Dietz 1981; Lerer et al. 1982) In general suitability of optimum conditions for bee activity varies from season to season, depending upon the geographical regions, time of the year, melliferous crops or species of bees. Abrol (2006a, b, 2007a, b, c) found that in case of *A. dorsata* temperature, light intensity, solar radiation; in case of *A. mellifera*, temperature, light intensity, solar radiation and RH; in case of *A. cerana* light intensity and in case of *A. florea* solar radiation and RH were the important factors. Abrol (2006a, b, 2007a, b) using path analysis of ecological factors did not support the widely held contention that changes in nectar sugar concentration are reflected by the spectrum of flower visitors (Corbet 1978a; Nunez 1977). According to him, this may be true under the experimental controlled conditions where all other factors are kept constant and bees have to respond to variations in one factor (nectar sugar concentration) only. Contrary to this under field conditions simultaneously operating multifarious factors, nectar sugar concentration, a biotic factor, like bee activity, is influenced by temperature and radiation as is evidenced from a high and significant correlation between them. Evidently, the resulting significant relationship between bee visits with nectar sugar is incidental under field conditions.

This reveals that nectar sugar concentration even though an important factor (Corbet 1978a) for which the bees have to fly and under controlled conditions its effect will be reflected in appreciable proportions, but under field conditions it also gets influenced by simultaneously operating other factors as the bee activity itself.

In between the commencement and cessation, *A. florea* activity was highest on flowers when temperature ranged between 25 and 38 °C and declined at higher temperatures (Abrol 2006a). Similar observations were made by Free (1993) who found that metabolic activity of insects increases as the temperature increases and they visit many flowers at that time. Similar results have been reported by several earlier investigators (Lerer et al. 1983; Burill and Dietz 1982; Nunez 1977).

Cirudarescu (1971) found that the number of insect visitors on lucerne varied directly with temperature and inversely with RH. Szabo and Smith (1972) reported that greatest foraging activity of *M. rotundata* occurred at 30 °C in bright sunshine

and declined at higher temperature in Hungary. Kapil and Brar (1971) recorded peak activity of *A. florea* on toria between 21 and 25 °C temperature and 50–57 % RH during November. In general suitability of optimum bee activity varies from season to season, depending upon the geographical regions, time of the year, melliferous crops or species of bees.

Abrol (2006a) studied the foraging behaviour of four honeybee species *A. dorsata* F, *A. mellifera* L, *A. cerana* F. and *A. florea* F. visiting litchi flowers (*L. chinensis* Sonn.) and found that maximum foraging populations of all the honeybee species were observed between 1100 and 1300 hours when the air temperature ranged between 23 and 34 °C, RH between 65 and 87 %, light intensity between 2,700 and 6,700 lx, solar radiation between 24 and 35 mW/cm² and nectar sugar concentration between 40 and 68 %. However, on cloudy/overcast days the pattern was altogether different. There was no well defined peak. Foraging populations were generally low in numbers and activity occurred only when ecological conditions within which foraging occurs were attained.

Neupane et al. (2006) found that the activity of *A. dorsata* foragers was more pronounced in all four plants (bottlebrush, litchi, citrus and summer squash) during the morning hours when air temperature was comparatively lower (19.3 °C at 7.30 a.m.) than that of afternoon hours (33.1 °C at 5.30 p.m.). The pronounced activity of the bees during morning hours was due to the early release of fresh pollen and nectar and suitable foraging temperature. The *A. dorsata* foragers forage for long distances by showing maximum foraging activity at a temperature between 25 and 35 °C, the distance and range being higher than the *A. mellifera* (Sihag 1998).

Sihag (2000a) found that *A. florea* started foraging when ambient temperature surpasses 18 °C and continues foraging until temperature approaches 43 °C. Maximum foraging activity is shown at 30–40 °C. These ranges are highest than those shown by *A. dorsata*, *A. mellifera* and *A. cerana*. In case of *A. dorsata* (Sihag 2000b) foraging commenced when temperature surpassed 16 °C and continued up to around 40 °C. Maximum foraging is shown at 25–35 °C. These ranges are lower than those shown by *A. mellifera*. Abrol (1992c, d) found that during winter, temperature acted as a stimulus for initiation of flight activity in honeybee *A. florea*, *A. dorsata*, *A. cerana* and *A. mellifera* whereas during summer, light intensity provided the minimum threshold. Woyke et al. (2003a) found that workers of *A. laboriosa* did not initiate flight activities below 10 °C and the flights were not performed during overcast days.

Foraging activity in the evening starts to decline at slightly higher temperature (Lundie 1925). The hive bee, *A. cerana* can forage at lower temperature than *A. mellifera* (Verma 1988). Foraging of bee is also greatly affected by changing temperature, e.g. no foraging occurs below 8 °C, some activity between 8 and 16 °C, optimum activity between 16 and 32 °C and reduce foraging above 32 °C (Roberts 1979, Anonymous 2003) but under some circumstances foraging may continue up to temperature of 42–48 °C. Verma and Dulta (1986) studied the comparative foraging behaviour of *A. mellifera* and *A. cerana* on apple bloom and found that activities of *A. cerana* started earlier in the morning and ceased late in the evening compared

to *A. mellifera*. The peak activities occurred between 0900–1130 hours and 1100–1329 hours in case of *A. cerana* and *A. mellifera*, respectively. An *A. florea* colony begins foraging between 0600 and 0730 hours and continues until dusk. Although foraging activity often diminishes during the hottest part of the day (Akratanakul 1977), foragers tend to keep close to their colony with a maximum range of 500 m. Although they prefer flowers whose outline appears subdivided (Dixit 1956), *A. florea* colonies have been found to collect more pollen from Compositae species than *A. cerana* and *A. dorsata* (Phadke 1968).

The individual temperature tolerance seems to be higher compared to *mellifera*, as concluded from the daily flight activity: In the morning, *florea* field bees start about 2 h later (at ambient temperature of 18 vs. 10 °C in *mellifera*), but continue also during the hours with temperatures higher than 40 °C, when *mellifera* stops all flight activities. Therefore, a significantly different daily flight pattern is the result.

A. dorsata was observed to forage and dance also during bright nights (Divan and Salvi 1965). The extrapolated position of the sun (Dyer 1985) is used for compass orientation, and not the moon. A proof of nocturnal activity is also the repeatedly observed attraction of *A. dorsata* bees to street lamps.

A. cerana is reported to be active at lower temperatures than *A. mellifera*. Field activities have been observed in Ussuria at 6–8 °C (Danilova 1960). The same was noticed in Kashmir (Shah 1980) and China (Oschmann 1961; Gong 1983). In Himachal Pradesh, *A. mellifera* is the more active species during the warm season and *A. cerana* during the cold one (Adlakha and Sharma 1974). *A. cerana* collects nectar from *Plectanthus* blossoms on cool autumn days, while *A. mellifera* does not (Sharma et al. 1980). In Japan *A. cerana* starts flying in spring 1 month earlier than times reversed. During cool morning hours, while *A. mellifera* remains still inactive, *A. cerana* is robbing their nests (Goyal 1974). Again, these behavioural differences might not be species-linked, but could be specific adaptations of certain ecotypes.

Altitudinal gradient affects the pollination and pollinator's foraging, e.g. humming birds are more effective pollinators at high elevations while bee at middle (Cruden 1972), and moths at middle and low altitudes (Cruden et al. 1976). The initiation of foraging activity of bees, *A. mellifera* and *A. cerana* was reported to be delayed and ceased earlier with increasing altitude (Verma and Dulta 1986). Fragmentation of natural habitat will disrupt the relationship between plants and its pollinators, e.g. two shrub species, *Acacia brachybotrya* and *Eremophila glabra* growing in linear vegetation received less pollen than conspecifics in nearby reserves (Cunningham 2000).

Wind affects the foraging activity of insects especially the flying ones. Wind velocity of 24–34 km/h adversely affects the foraging behaviour of bees (Lundie 1925; Verma 1990). Downwind movement of floral odour or upwind flight due to wind greatly influenced directional foraging of bumblebees (Woodell 1978). Moisture also affects the activities of insect pollinators like any other organism. Bumblebees continue foraging during light precipitation (Bruggeman 1958). Cloudy and rainy environment may restrict the activity of syrphid (Levesque and Burger 1982) and bees (Cruden 1972), respectively.

11.8.3 Size Related Foraging in Relation to Weather Conditions

The large size of *A. laboriosa* workers is almost certainly one of the major adaptations that has enabled this species to survive in temperate climates while the other open-nesting honeybees are confined to the tropics and subtropics. *A. laboriosa* workers are able to forage at ambient temperatures at least 5–6 °C lower than the minimum ambient temperature at which *A. dorsata* workers can fly (Dyer and Seeley 1987, 1991; Underwood 1991). This has apparently been accomplished largely through an increase in body size, without resort to creating a higher-powered bee and without a disproportionate increase in thoracic mass, such as seems to have been the case with *A. cerana* in Nepal.

11.8.4 Factor Compensating Mechanisms

Abrol (1985) found that resultant foraging activity in bees may be a consequence of several interacting factors in which value of one factor with higher values may compensate the lower values of the other factors. He found that *A. florea* commenced foraging activities where a combination of 19.5 °C temperature, 87 % RH, 1,700 lx light intensity and 64 mW/cm² was attained, however, on another day activity was interrelated at higher values temperature when other factors were comparatively less in intensity. This implies that higher values of temperature can compensate the lower values of others and this trigger the activity, while in the former case higher values of light intensity and solar radiation could compensate the lower values of temperature and result in the initiation of the activity.

Abrol and Kapil (1986) reported similar compensating mechanism operating at the commencement of pollination activity of *M. lanata* in which lower values of temperature were compensated jointly by higher values of light intensity and solar radiation and vice-versa. A solar phenomenon has been reported by Szabo and Smith (1972) who found that *M. rotundata* foraged at 1,075 lx when the temperature was 25 °C but needed 6,450 lx at 17 °C. Foraging in honeybees is under short duration turn-key control of the physical environment. Each bee species is guided by the specific ecological threshold for normal foraging activity whose maintenance differ inter- and intraspecifically depending on their adaptability (Abrol 1985, 1987a, b, c).

11.8.5 Ecological Threshold of Nectar Secretion and Foraging Activity of Honeybees

Co-evolutionary adaptations have brought a close correlation between plants and their pollen vectors in a manner that functional activity of both more often synchronizes. Each bee has its ecological threshold for initiation of field activity, until surpassed, activity is limited (Abrol and Kapil 1986). In plants also a characteristic rhythm of

nectar secretion occurs with peak periods of nectar production usually synchronizing when the conditions are optimum for bee activity (Corbet 1978a, b). Abrol (1985) reported that in *Apis* species such as *A. dorsata* and *A. florea* onset of flight activities occurred at 15 °C under subtropical conditions of Hisar during winter months. Abrol (1986b) found that in flowers of *B. juncea* nectar secretion occurred between 12 and 15 °C thereby indicating that 12–15 °C as a minimum threshold temperature for activation of enzymatic machinery to secrete nectar. This overlap of activity threshold of two different groups makes them interdependent to rely on each other, one for energy reward and another for reproduction and perpetuation of its race. Abrol et al. (1988a) reported that under temperate conditions of Kashmir, India where no nectar secretion was observed in flowers of *Eriobotrya japonica* below 8 °C which began at 10 °C coinciding with initiation of flight activity of *A. cerana*. Similarly, threshold temperature below which no nectar is produced has been reported for many plants, Boss wood (*Tilia Americana*) begins to secrete at 18 °C (Desmuth 1933), bird cherry (*Prunus laurocerasus*) at 18–20 °C, cucumber (*Cucumis sativa*) at 17–21 °C (Collision 1973) and *B. juncea* at 12–15 °C (Abrol 1986b). Evidently, physiology of plants and their pollen vectors have synchronized in a manner that functional activity of both most often synchronizes for the benefit of two.

11.8.6 Sequence and Timing of Bee Visits

The visits of the bees to the flowers depend largely upon the energy requirement reward system and occur in a very predictable and ordered manner (Schlising 1970; Heinrich 1975a; Murrell and Nash 1981). Parker studied the diurnal rhythm of bee visitation on sunflower and found that most of the oligolectic bees (*Andrena*, *Megachile* species) visited the flowers earlier in the day than did the polylectic bees (*Apis*, *Bombus* etc). Linsley and Cazier (1970) have recognised five temporal categories: matinal, diurnal, late afternoon, crepuscular and nocturnal. Abrol (1987a, b) found that larger bees like *A. dorsata*, *A. mellifera* and *A. cerana* with higher energetic demands and superior thermoregulatory capabilities visited flowers early in the morning when the caloric reward is more and competition with other pollinators is minimal. Whereas, the smaller ones such as *A. florea* with relatively low energy requirements and poor thermoregulatory capabilities visited flowers in the late morning hours, when energy expenditure due to prevailing higher temperature was minimized.

11.8.7 Foraging as a Function of Species Specific Adaptation

Several species of highly social *Trigona* (Johnson and Hubbell 1974, 1975) and *A. mellifera* (Sakagami 1959; Schaffer et al. 1979) specialize on large productive flower clusters. The large size of their colonies (Michener 1974) results in large

pollen and nectar requirements for colony maintenance and growth, which favour the efficient exploitation of large resource patches. The ability of many stingless bees and of honeybees to communicate with their nest mates and to lead or direct them to floral resources facilitates this specialization. Despite controversy over the ability of recruits to utilize the information in the bee dance (Gould 1976; Rosin 1978; Wenner 1972), the excellent recruitment ability of *A. mellifera* is undisputed. In contrast, some bee species may be low-density specialists in their flower foraging (Johnson and Hubbell 1974). Other species forage randomly on available flower patches. The forager densities of several species of halictines, for example, were positively and linearly related to the density of *Convolvulus arvensis* flowers in field samples (Waddington 1976). Abrol (1998a, b) found that under similar set of climatic conditions the responses of *A. cerana* and *A. mellifera* were absolutely different. *A. cerana* started flying earlier in the morning than *A. mellifera* did, and also at a lower temperature, light intensity and solar radiation and also worked for longer periods. Solar radiation directly affected the activity of *A. cerana*, and solar radiation and light intensity directly affected that of *A. mellifera*. Forbes and Cervancia (1994) also reported similar findings in case of *A. cerana* and *A. mellifera* in Majajjay, Philippines.

Abrol (2006a) studied the foraging behaviour of four honeybee species *A. dorsata* F., *A. mellifera* L., *A. cerana* F. and *A. florea* F. visiting litchi flowers (*L. chinensis* Sonn.) and found that all the four honeybee species differed in their responses to environmental factors prevailing under similar set of conditions depending upon physiological adaptation of each honeybee species. Of all the factors studied, temperature, light intensity and solar radiation were the three important factors whose influence on foraging population was more pronounced.

11.9 Competition for Floral Resources

Researchers have recently obtained evidence of competition between bee species in natural situations. Inouye (1978) studied two species of bumblebees that were foraging primarily on different flower species in Colorado. When he removed one bee species, one flower species was left unused. Foraging patterns of the other bee species shifted to include more of the abandoned flower species. Morse (1977) noted that workers of two bumblebee species were partitioned along sprays of golden-rod, *Solidago canadensis*. Apparently, *Bombus ternarius* workers avoided the larger *B. terricola* workers (Morse 1977). Morse (1978) recorded interspecific displacement of bumblebee workers on roses, as well. From his data on nectar depletion and resource partitioning in Maine, Heinrich (1976) inferred that four bumblebee species competed for nectar. Johnson and Hubbell (1974, 1975) reported aggressive defence of favourable resource sites by certain stingless bees. The spacing of nests of these bees may reflect competition for floral resources (Hubbell and Johnson 1977). *A. cerana* is sympatric in distribution and can co-exist with the two other species of

Asiatic honeybees, *A. dorsata* and *A. florea*, without any adverse ecological consequences. Chahal et al. (1986) found that *A. florea* showed aggressiveness towards *A. mellifera* both at hive and on flowers.

Partap (2000a) studied the foraging competition between *A. cerana* and *A. mellifera* on four crops blooming simultaneously and found that *A. cerana* foragers were less abundant when *A. mellifera* bees were in the plots in all the crops. Removal of *A. mellifera* colony from the plots resulted in reversal of the abundance trend among both the species. After removal of *A. mellifera* the number of *A. cerana* foragers increase significantly from 12.6 to 20.8 in mustard, 12.3–18.3 in broad leaf mustard, 18.4–28.3 in cauliflower and from 9.7 to 16.2 in radish. Evidently, presence of *A. mellifera* in the field reduces the number of *A. cerana* bees in the field. The two species try to displace each other rather than visiting/pollinating flowers. It is, therefore, inferred that the presence of both species adversely affect the pollinating efficiency of both the bees. He further found that removal of *A. mellifera* resulted in significant increase in time spent per flower, number of flowers visited per minute, weight of pollen load, number of pollen collectors, but after its removal number of pollen loads from other crops decreased significantly. Numerous interactions among the species were noted, small individuals generally attacking larger ones. *A. dorsata*, however, was attacked only by *A. cerana*, never by the other two species. In case of *A. florea*, their small size does not deter from showing aggression towards other *Apis* species at a food source. When foraging at dishes containing sugar syrup, *A. florea* was as successful (or more so) than *A. cerana* and *A. dorsata* in maintaining its station there and inducing the departure of bees of other species. However *A. florea* and *A. cerana* workers sometimes forage together at an artificial food source without showing aggression.

11.10 Resource Partitioning

In a mutualistic relationship with flowering plants, honeybees gather nectar and pollen from blossoms. Yet not all honeybee species exploit the same floral resources. This is partly reflected in geographical variation, because honeybee species or subspecies often live in allopatric ranges which contain different types of vegetation (Schneider and McNally 1992, 1993). In cases of sympatry, resources may be partitioned in other ways. For example, resources may be partitioned based on size. Sharma et al. (2000) studied resource partitioning in *A. mellifera* and *A. cerana* and found that of the 23 plant species studied *A. mellifera* and *A. cerana* avoided competition by visiting the plants in differing intensities. During spring *A. mellifera* preferred *Rubus ellipticus* (7.85 bees) and *Prunus armeniaca* (7.6 bees) whereas *A. cerana* was more active on *Malus domestica* (9.91 bees). Partap et al. (2000a) found that *A. cerana* workers started foraging earlier in the morning (0731 hours on peach and 0812 hours on plum) compared to *A. mellifera* worker bees that started foraging at 0801 hours at peach and 0837 hours at plum. In the evening, *A. mellifera* stopped earlier (1735 hours on peach and 1702 hours on plum) compared to *A. cerana*

Table 11.2 Tongue length and body weight of different honeybee species

Bee species	Tongue length (mm)	Reference	Body weight (mg)	Reference
<i>A. florea</i>	3.31	Dyer and Seeley 1987	22.6 23.4	Underwood 1991 Abrol 1985
<i>A. dorsata</i>	6.45 6.728	Dyer and Seeley 1987 Sakagami et al. 1980	118.0 123.0	Underwood 1991 Abrol 1985
<i>A. cerana</i>	5.40 4.39–5.53	Dyer and Seeley 1987 Mishra and Kumar 1997	55.0 77.0	Underwood 1991 Abrol 2007a, b, c
<i>A. mellifera</i>	6.11 5.7–7.2	Dyer and Seeley 1987 Mishra and Kumar 1997	85.0 113.0	Underwood 1991 Abrol 2007a, b, c
<i>A. laboriosa</i>	7.05	Sakagami et al. 1980	165.4	Underwood 1991

(1806 hours on peach and 1751 hours on plum). Sihag (2000a) reported that *A. florea* starts foraging when the ambient temperature surpasses 18 °C and continues foraging until ambient temperature approaches 43 °C. Maximum foraging activity is shown at 30–40 °C. These ranges are highest than those shown by *A. mellifera* and *A. dorsata*. In case of *A. dorsata* (Sihag 2000b) foraging commenced when temperature surpasses 16 °C and continues to around 40 °C. Maximum foraging activity is shown at 25–40 °C. These ranges are lower than *A. florea* but higher than *A. mellifera*. Gupta and Reddy (1992) found that foraging activity of *A. mellifera* and *A. cerana* on wild cherry *Prunus puddum* Roxb. peaked during 1100 and 1400 hours, respectively. Body size and tongue length plays a predominant role in determining the resource partitioning of the bees (Table 11.2). For example, Oldroyd et al. (1992a, b) found evidence suggesting that pollen resources were partitioned among *A. dorsata*, *A. cerana*, *A. florea*, and *A. andreniformis* based on bee size with the larger two species using the richest pollen resources.

El Shafie et al. (2002) studied 21 pollen sources for the honeybee *Apis mellifera sudanensis* and the dwarf honeybee *A. florea* between early December and late March and found some hints of competition between two species. However, further studies showed that *A. mellifera sudanensis* and *A. florea* do seem to co-exist. This co-existence is based on different daily rhythms of pollen collection. *A. mellifera* collected pollen of *Acacia seyal*, date palm, and onions early in the morning (and partly in the late afternoon), while *A. florea* started pollen collection mostly later in the morning and ended it earlier in the afternoon. But in contrast to *A. mellifera*, *A. florea* was collecting pollen all day without interruption, even at very high air temperatures. Niche overlap (concerning the times of visits to flowers) between the two bee species was very low in date palms, and of medium importance in *A. seyal*. But it is remarkable that in total, *A. florea* is always present in higher numbers than *A. mellifera sudanensis*, on flowers. Gupta et al. (1984) found that *A. cerana indica* started foraging on *Plectranthus* in the morning at 0600 hours, whereas *A. mellifera* was seen only after 0700 hours. Similarly, in the evening, *A. mellifera* stopped working after 1700 hours and *A. cerana indica* continued to forage until 1800 hours. During these observations both species of the bees collected only nectar from the flowers of *P. rugosus*. Even though other good sources of pollen were available to bees, no other nectar sources were observed in the area. Neupane et al. (2006) found

that *A. dorsata* preferentially concentrated their visits to litchi flowers in the early morning hours and then declined towards the later part of the day significantly during the entire periods of flowering. The bees collected nectar more preferentially from bottlebrush, litchi flowers and pollen from bottlebrush, citrus and summer squash flowers showing a distinct resources partitioning. However, the flowers of radish were more preferentially foraged by the worker bees of *A. cerana* and *A. mellifera* for pollen collection showing a distinct foraging preference by different honeybee species. Several researchers have reported similar findings. Rao and Lazar (1983) in a study of bee behaviour and pollination in onion revealed that *A. dorsata* never visited the flowers of onion during the entire period of its blooming, while *A. cerana* and *A. florea* were observed collecting nectar. Verma and Pratap (1994), Partap and Verma (1994), and Partap et al. (2000b) reported that cauliflowers were more attractive to *A. cerana* than radish.

Partap (2000b) reported that *A. cerana* started their foraging activities on sweet orange *Citrus sinensis* earlier in the morning at 0600 hours and ceased late in the evening at 1835 hours, the total duration of foraging was 1205 hours and the average duration of foraging was 22.6 min. Peak foraging activity was observed between 1000 and 1200 hours. A nectar collector on an average visited 5.5 flowers/min and a pollen collector visited 8.2 flowers/min. Partap et al. (2000b) found that *A. cerana* workers mostly worked flowers from top and collected mainly pollen indicating its efficiency in fruit crop pollination. Top working pollen collectors are considered to be better pollinators than side workers collecting nectar. Singh (2008) reported that *A. cerana* started their foraging activities early in the morning at 0614 ± 0004 hours and ceased late in the evening at 1728 ± 0011 hours. The total duration of foraging activity was 1000 hours and the average duration of foraging trip 4.5 ± 0.146 min. Two peaks of foraging activities were observed from 0830 to 1030 hours and from 1130 to 1330 hours when the temperature of experimental field ranged between 20 and 21 °C.

11.11 Resource Partitioning Between *Apis cerana* and *Apis nirocineta*

Sometimes resources are partitioned when separate species forage at different times of day. In Bhubaneswar, India, *A. cerana* and *A. dorsata* were most active on niger plants at 1100 hours. *A. florea* in contrast, was more active in the afternoon (Panda et al. 1993). Daily temporal differences in foraging may be related to temperature constraints. In apple orchards in India, *A. cerana indica* and *A. mellifera* showed different daily peak times of foraging. *A. cerana indica* foraged mostly between 0900 and 1130 hours, when the temperature was between 15.5 and 21 °C. *A. mellifera* peak foraging occurred between 1100 and 1330 hours, when the temperature ranged from 21 to 25 °C (Verma and Dulta 1986; Verma 1995). *A. cerana* also began foraging earlier in the morning and ended later in the day than *A. mellifera* (Verma 1995).

In addition to different times of foraging, food resources may be partitioned spatially. For example, species may forage on different strata. Rinderer et al. (1996) found that *A. cerana* and *A. koschevnikovi* in Sabah, Borneo, foraged more frequently at the tops of the yellow flame tree (*Peltophorum pterocarpum*) than at the middle or bottom levels. *A. dorsata* foraged equally at the top and middle of trees, while *A. andreniformis* foraged equally at all strata. These results suggest that species demonstrate stratum fidelity. Alternatively, nectar and pollen production may be more pronounced at the top and middle of trees, and certain bees (e.g. *A. cerana* and *A. koschevnikovi*) may competitively exploit the best resources. Roubik (1993) investigated the stratal associations of 20 bee species from ten genera in Panamanian forests. He found large variation in stratum association; most bees foraged at both heights or came consistently to the lower traps. Only two nocturnal bee species foraged mainly in the high canopy, and some diurnal species actually seemed to avoid the upper canopy (Roubik 1993). In the Malayan forest, instead of solitary bees, *A. cerana*, *A. dorsata*, and a few *Trigona* species seemed to be the main pollinators in the upper canopy (Appanah and Kevan 1995).

Verma and Chauhan (1985) observed insect pollinators on apple trees and found that 40.6 % of them foraged between 2 and 3 m from the ground. The rest foraged in roughly equal numbers above 3 m and below 2 m. The higher numbers on the middle branches may indicate stratified foraging, but alternatively may merely reflect a greater number of flowers on the middle branches. Abrol (1988) reported that *A. cerana* foraged in large numbers on middle branches of loquat, *E. japonica* about 3 m above ground compared to lower or upper branches under temperate conditions of Kashmir. The bumblebees on the other hand were observed in large numbers on topmost branches above 3 m from the ground.

Differences in proboscis lengths may be one way in which resources are partitioned between *A. cerana* and *A. nigrocincta*. Bumblebees with proboscis of different sizes have been found to utilize flowers with corresponding corolla lengths (Ranta and Lundberg 1980). In morphometric analyses of *A. cerana* and *A. nigrocincta*, samples of each collected from locations only 12 km apart had significantly different proboscis lengths ($P < 0.0001$) (Hadisoesilo et al. 1995). *A. cerana* had a mean proboscis length of 4.7 mm, and for *A. nigrocincta* the mean length was 4.995 mm (Hadisoesilo et al. 1995; Hadisoesilo 1997). These two species may handle some of the same flowers with differing amounts of ease because of their different proboscis lengths. Extensive comparisons have been made between Africanized and European honeybees, which belong to the same species (*A. mellifera*) and freely interbreed but which are nonetheless geographically distinct races (Pesante et al. 1987a). Comparisons of foraging by these two species are of particular interest because of the overwhelming success of *Apis mellifera scutellata*, the African bee, in South and Central America (Seeley 1985). Danka et al. (1990) found only minor differences in foraging flight characteristics between the two races. However, Africanized bees made more visits for pollen than European bees (Pesante et al. 1987a), and Africanized bees collected more pollen during the dry season (Pesante et al. 1987a, b). In addition, European and Africanized bees may have different diurnal foraging strategies; both groups started out with similar foraging patterns early in the morning,

but European bees had more foragers for the rest of the day. More information on foraging preferences of the four *Apis* species and on competition between them is urgently needed particularly for *A. florea* and *A. mellifera*. A number of observations are available regarding different flower preferences between the two species. *A. cerana* (from Japan) is reported to visit a greater variety of plants, including wild species, while *A. mellifera* foraged mainly on *Trifolium* and *Brassica* (Miyamoto 1958). In Himachal Pradesh, the main nectar source of *A. cerana*, *Plecthranthus* sp., is neglected by *A. mellifera* foragers (Goyal 1974; Ahmad 1982). Honey collected from *A. cerana* bees (originating from China) in Germany, showed differences in the pollen spectrum compared to *A. mellifera* honey collected simultaneously at the same location (Vorwohl 1968).

11.12 Comparison of Foraging Between *Apis cerana* and *Apis nigrocincta*

The similar size and resource needs of *A. cerana* and *A. nigrocincta* suggest that these species could share similar ecological niches, yet in zones of sympatry they seem to occupy different habitats. Bakker (1999) found that *A. cerana* forages in disturbed habitats and *A. nigrocincta* forages more extensively in forested areas. In *A. cerana*, dancing scouts indicated distances from 10 to 1,420 m, and the average dances for final destinations ranged from 99 to 780 m. Dances of *A. nigrocincta* scouts ranged from 75 to 2,340 m, and final destinations averaged from 140 to 1,920 m indicating that *A. nigrocincta* travelled farther than *A. cerana* when scouting for new nest sites.

Bakker (1999) reported that foragers of *A. cerana* and *A. nigrocincta* concentrated on different pollen resources, suggesting that resource partitioning may be happening on a different level but the reasons for preferences for different kinds of pollen are as yet unknown. A comparison of *A. cerana* and *A. nigrocincta* foraging activity throughout the day revealed large variation from day to day both among and between colonies. Resources do not seem to be partitioned by differences in time of foraging; however, slight differences in time of foraging may result from each colony exploiting different resources. He further found that *A. cerana* and *A. nigrocincta* have dance curves with equal slopes, but *A. nigrocincta* started doing a waggle dance at a farther distance from the hive (25 m, compared to 10 m for *A. cerana*). *A. nigrocincta* also had a wider flight range than *A. cerana*, both in distances travelled while foraging and distances of nest sites investigated by swarm scouts, but both species had relatively short foraging ranges. A wider flight range could give *A. nigrocincta* an advantage over *A. cerana*, enabling the former to scout farther for food and for nesting sites. However, he did not find any significant difference in foraging locations of two species.

11.13 Learning and Memory in *Apis cerana*

The honeybee offers several advantages as a model organism for studying learning and memory, including a relatively simple brain structure, social organization allowing easy rearing, and a complex behavioural repertoire, which is readily

manipulated. Although a honeybee has only a tiny brain that has only about 1 million nerve cells in it (only one hundred-thousandth of the human), it has an amazing ability to learn and remember tasks and objects (Menzel 1990, 2001a, b; Giurfa et al. 1996, 2001; Zhang et al. 1996, 1999, 2000, 2004; Giurfa 2007). Learning and memory performances in honeybees were quantified in a colour learning experiment by Menzel (1967, 1968) for the first time; this initiated an interest in honeybees' learning and memory research. Since then, it has been found that honeybees can not only learn to distinguish different colours and orientations (Hateren et al. 1990; Zhang and Srinivasan 1994), but can also extract abstract concepts from visual patterns (Giurfa et al. 2001; Zhang et al. 2005; Pahl et al. 2007; Gross et al. 2009; Avargues-Weber et al. 2011; Avargues-Weber et al. 2012). However, most research attention in this field has centred on Western honeybees, and as a result, the learning and memory capabilities of most tropical bees have not been properly investigated. Some research has recently started on stingless bees in South America (McCabe et al. 2007; McCabe and Farina 2009, 2010), but Asian bees still need to be characterized. As prototypical honeybees of the East and the West, *A. cerana* and *A. mellifera* are two important honeybee species that are widely bred and studied. Recent work on the two species has revealed that both geographical isolation and long-term evolutionary divergence are responsible for differences between the two species in key biological characteristics including shape, individual development and living habit, etc. (Chen 2001; Zeng 2009). However, there have been hardly any direct comparative studies on their learning and memory capabilities up to now.

Qin et al. (2012) stated that the honeybee is an excellent model organism for research on learning and memory among invertebrates. Learning and memory in honeybees has intrigued neuroscientists and entomologists in the last few decades, but attention has focused almost solely on the Western honeybee, *A. mellifera*. They showed for the first time that the learning and memory performance of *A. cerana* is significantly better on both colour and grating patterns than that of *A. mellifera*. They speculate that this can be largely explained by the different habitats of these two honeybee species, where there are different climates, weather conditions, food sources and the onsets of flower blooming. The Eastern honeybee, *A. cerana*, which is apparently better adapted to hot climates and able to avoid the oriental hornet, a serious predator, and is also good at collecting sporadic nectar flowers, is distributed from Afghanistan to Japan and Southeast Asia to the Wallace line; the Western honeybee, *A. mellifera*, on the other hand, is about one-third larger in size and is better adapted to extended periods of cold weather (Butler 1975; Dietz et al. 1986). According to reports, most of the European mainland is plains, with an area of more than 60 %, and its mountainous area is less than 2 % (Fan and Zhou 2005). However, China, the main habitat of *A. cerana*, is primarily a mountainous region (more than 75 % of the country) (Li et al. 2008; Cheng et al. 2009). Because of the complex terrain and variable climate, the mountainous area is a very harsh environment for *A. cerana*. Furthermore, mountainous areas have more rain and fog, more dispersed nectariferous plants and more natural enemies compared with plain areas. Consequently, the survival pressure on *A. cerana* may be heavier than that on *A. mellifera*, so we infer that *A. cerana* needs to have a greater learning and memory capability because of the complex environment. Moreover, this environment

may also result in more opportunities for *A. cerana* to evolve in ways to adapt to the complex living environment. In conclusion, the pressures of survival may play an important role in the phenomenon that *A. cerana*'s performance in learning and memory is superior to that of *A. mellifera*.

Qin et al. (2012) found that there were significant differences between *A. cerana* and *A. mellifera* in learning and memory performance on colour and orientation learning and that the performances of *A. cerana* were significantly better than that of *A. mellifera*. As shown by Chen (2001), *A. cerana* has a better sense of smell than *A. mellifera*. A recent study has revealed that in mixed colonies of *A. cerana* and *A. mellifera*, the two species can understand each other's 'dance language' (Su et al. 2008). In comparison to *A. mellifera*, however, *A. cerana* can decode the 'dance language' more accurately and quickly. This demonstrates that *A. cerana* may have a stronger learning and memory capability than *A. mellifera*. Our result showing that the performances of *A. cerana* are significantly better than those of *A. mellifera* is in agreement with them.

11.14 Foraging in Relation to Flavour, Taste and Colour of Flowers

The actual recognition of source of food or nectar is influenced by various factors such as colour, flavour and taste. For example bees can distinguish green, blue, white, yellow, violet flowers but are insensitive to red flowers. The bees can also discriminate between different concentrations of sweetness and varying salt content in flowers. If flowers of different concentrations are blooming simultaneously, the bees will prefer those which have higher sugar content. Once the bees have started to work on a certain source for nectar or pollen, they continue to visit it and seldom visit more than one or two species on a single trip. Bees also drink water and they like stagnant water rather than fresh. If pinch of salt is added to the fresh water it also becomes attractive. It is again with the sense of smell, that bees can recognize their nest mates. If by mistake a bee of other colony intrudes, it is recognized and killed at once. Strangely enough, if the stranger is laden with nectar or pollen it is not driven away or killed. Surprisingly, drones of all hives are allowed free entry.

Foraging behaviour of *A. florea* was studied on *P. grandiflora* flowers having different patches of straw yellow, dark yellow, purple and red flowers (Abrol 2003). Maximum foraging populations was observed on straw yellow flowers followed by dark yellow, purple and red flowers, respectively. Earlier, Faegri and van der Pijl (1971) have reported that *A. mellifera* preferred yellow, blue and purple flowers. Sharma et al. (1999) found that pink, red and blue colours attracted more honeybee and bumblebee pollinators; black and green were less effective than the control (white).

11.15 Nocturnal Foraging

Dyer (1985) observed that in case of Asian honeybee *A. dorsata*, although the moon's illumination is essential for nocturnal flight, the moon itself is ignored for orienting the dances. Rather, bees probably use the sun's position as a reference point for

their dances, even though the sun is below the horizon. This ability may involve an extension of the mechanism that honeybees employ to find the sun on overcast days.

Rao et al. (2008) found that *Pterocarpus santalinus* blooms massively in the dry season. The flowers open at mid-night and are bright yellow in colour. They are homogamous and are visited by the rockbee *A. dorsata* at moonlit nights or otherwise at dawn and up to 0730 hours. The natural habitat of *P. santalinus* has a hot dry climate, and the nocturnal flowering and foraging of the rockbee at that time during moonlit nights appear to be an adaptation to avoid the adverse effects of daytime high temperatures.

11.16 Flight Pattern

The flight of *A. cerana* resembles that of a fly—rapid, hasty, and unpredictably zigzag—compared to the steady, clumsy flight of European *mellifera* bees (Kellog 1941; Lindauer 1956; Sakagami 1959). This behaviour helps in escaping from flying predators, like hornets and bee-eating birds. It has to be investigated, however, whether this flight pattern is species-specific and whether some tropical *mellifera* races show the same behaviour. When bees were dislocated 50 m, the speed of homing was found to be shorter for *A. cerana indica* (192 s) than for *A. mellifera ligustica* (295 s; Atwal and Dhaliwal 1969).

11.17 General Flight Activity and Foraging Range

Bees are the most important pollinators and understanding the scale at which they forage has important ecological implications and conservation applications (Axelrod 1960; Bawa 1990).

Bees are the primary pollinators for most ecological regions of the world. Flight activity of honeybees is an index of their foraging potential for pollination and honey production. Bees recognize their hive and surroundings and can return to their hive after completing the field activities. If the hive is shifted by more than 3 ft from its original position, the bees returning from their foraging trips get confused and fail to locate new location. They will stay on the old spot and some of them may eventually die. The bees have a tendency to fly in one or two major lines of flight called as beeline. Bees generally prefer foraging near hive. The longer flights are generally avoided, because the food required for such an effort will be more than the nectar gathered. Their foraging distance strongly influences the sexual reproduction of most flowering plants and can determine the genetic structure of plant populations (Campbell 1985; Waser et al. 1996). Bee foraging distance also affects agricultural production. Many bees that pollinate crops nest in natural habitats and forage on crops within their daily travel distance (Ricketts 2004). Foraging distance therefore determines the spatial scale at which bees can provide pollination services to crops (Kremen 2005). Foraging range of pollinators provides information regarding their intrinsic capabilities in gathering nectar and pollen resources (Cherian and Mahadeven 1945a, b; Cherian et al. 1947; Eckert 1955; Dhaliwal and Sharma 1972, 1973,

1974; Naim and Phadke 1972; Kapyla 1978; Naim 1984; Abrol and Kapil 1986; Abrol 1988c; Abrol et al. 1988a, b, 1989; Abrol 1993a, b, c, 2008; Dyer and Seeley 1991). The foraging range of bees depends largely on their body size and energy needs (Abrol 1986a; Abrol and Kapil 1986), yet is also determined by the quality of reserve fuel carried when bees are in flight. The smaller bees with low energy requirements and little reserve fuel may hardly risk foraging large distances (Hocking 1968). Abrol (1988a) reported *A. florea* workers to forage up to 150 m from their nesting sites compared to *Megachile flavipes* and *Megachile nana* which foraged up to 250 and 150 m, respectively. Bees generally visit flowers within a radius of 1–3 km. *A. cerana* normally forages 1–1.5 km while as *A. mellifera* may forage up to 3 km, but if the food sources are limited and the competition is more the foragers may go long distances. The foragers of *A. cerana* have been reported to fly 600–1,040 m while that of *A. mellifera* from 3 to 14 km.

Normally the bees prefer to forage near their nesting sites. Decrease in return from increased distances from nesting sites may be related to partial familiarity with an area. Bacon et al. (1965) reported that bees extend their foraging range when the flowers become sparse. Evidently, the reasonable flight range of bees may be less than the reported values in literature.

In a flight room *A. cerana* colony from Pune (India) with permanent light and feeding within the hive showed 5.5 times as much flight activity relative to the number of bees of a colony of *A. mellifera carnica* (Preuhs 1971). Lindauer (1956) was not able to train *A. cerana* workers in Sri Lanka farther than 750 m from the hive. During these experiments the flight distance of bees collecting on natural sources did not exceed 300 m. A main foraging distance on cauliflower (75 % of the bees) of up to 400 m, on barberry 600–700 m, was found in Punjab, India (max. distance 4,900 m, or 1,100 m; Dhaliwal and Sharma 1973). The uphill foraging distance was much shorter (300 m) than in flat country (Dhaliwal and Sharma 1974). In Kashmir, *A. cerana* bees were observed to collect pollen from *Crocus sativus* at a distance of 3.75 km (Shah and Shah 1980). *A. florea* bees are the most important pollinators of field crops in the plains of India and Pakistan. Atwal (1970) and Sihag (1986) observed 73–84 % *florea* bees among the insects visiting a broader array of plant species (Koeniger and Vorwohl 1979; Whitcombe 1981, 1984) than larger species, thus making up for the short flight range, which usually does not exceed 500 m (Lindauer 1957; Targari 1971).

Dhaliwal and Sharma (1973) found that maximum foraging range of *A. cerana* on barberry and on cauliflower was 900 m and 1100 m, respectively. However, maximum distribution of foragers was within 400 m in cauliflower and 600–700 m in barberry. Sharma (1972) found that under similar conditions *A. mellifera* worked within 1,200 m on mustard flowers, however few individuals could be found up to 5 km. However, few workers have found that effective foraging range from hive is within 800–2,000 m and in exceptional cases foragers have been recovered up to 8–10 km from the hive (Knaffl 1953; Eckert 1955; Peer 1955; Kettner 1967). The relatively small flight range of *A. cerana* may be related to its body size is perhaps responsible for low honey yields and high incidence of absconding. Ichikawa et al. (2000) reported that foraging range of *A. mellifera* varied during seasons on different crops depending upon the availability of forage from 0.9 to 2.5 km, in central district of Tokyo, Japan, however, maximum foragers were available within 100 m when resources were abundant.

It is one of the most common assumptions that honeybees only forage close to their hive. However, in certain instances, they have been reported to forage up to 12 km or more from the hive. von Frisch (1967), the discoverer of the honeybee dance language, was able to calibrate the dance language at distances of up to 9.5 km. The greatest estimate came from Eckert who studied bees in the desert in the western United States and reported that they flew up to 13.5 km from the hive.

Foraging distances will vary with environmental conditions, such as the density and distribution of floral resources and the general physical resistance of the different habitats to flight (e.g. Ricketts 2001). Cresswell et al. (2000) provides evidence that the quantity and quality of available floral resources also affect foraging distance. For example, both honeybees (*A. mellifera* and *M. rotundata*) have been observed to increase their foraging distances with the increase in the distance to high-reward resources (Beekman and Ratnieks 2000; Bacon et al. 1965) and with resource scarcity (Steffan-Dewenter and Kuhn 2003). Honeybees will fly farther to get some resources than to acquire other resources (Gary et al. 1972), and their foraging distance varies as a function of landscape context (Steffan-Dewenter and Kuhn 2003).

The dwarf bee *A. florea* have a short flight range, often hardly reaching 100 m from the nest. The maximum distance it can fly from the nest for foraging is often less than 750 m. The dwarf bee is able to survive in very hot and dry climates with ambient temperatures reaching 50 °C or more. Rockbees can forage even during moonlit nights (Diwan and Salvi 1965). Its flight range is more than 5 km (Koeniger and Vorwohl 1979). In the normal forage conditions they have been observed to visit sources 2–3 km away from the nest.

11.18 Flight Intensity of Foraging Bees

Wing beat frequency is an expression of flight intensity and is an important parameter not only from the aerodynamics and bioenergetics points of view but also helps in assessing the pollinating efficiency of bees (Puranik et al. 1977; Heinrich 1975a, b). The wing beat frequency is generally species specific. In general, smaller insects have higher wing beat frequency than the larger ones (Sotavalta 1952). Generally the insects with smaller wings have higher wing beat frequency than those with long or heavy wings. Smaller the surface area of the wings more rapidly the wings must beat to keep the animal in flight (Kammer and Heinrich 1974). Wing beat frequency is a function of body parameters. Besides, the body parameters, wing beat frequency is also influenced by environmental temperature in all the insects except hymenoptera (Reed et al. 1942).

Abrol (1985) found that wing beat frequency of *A. florea* weighing between 12 and 19 mg ranged between 92 and 106 cps with an average of 101 cps and that of *A. dorsata* weighing between 106 and 129 mg was between 111 and 116 cps with an average of 113.95 cps. Interestingly, smaller bees on the basis of per gram body weight were found to have higher values of wing beat frequency than the larger ones. It was found that *A. florea* weighing $12\text{--}19 \times 10^{-3}$ g had wing beat frequency 101 cps in contrast to *A. dorsata* weighing $106\text{--}127 \times 10^{-3}$ g which had 113.95 ± 0.60 cps. *A. florea* per gram body weight exhibited 6,688.79 cps compared to *A. dorsata* where number was found to be 1,162.16 cps/g/body weight—more than

five-fold a difference. These results suggested that smaller bees exhibited higher wing beat frequency than the smaller ones.

A. cerana from the plains of northern India (Punjab) showed higher frequencies than yellow *A. mellifera* (presumably *A. mellifera ligustica*) from Europe: 306 vs. 235 wing beats/s for workers and 283 vs. 225 wing beats/s for drones (Goyal and Atwal 1977). These frequency differences could well be a mere consequence of wing size differences.

11.19 Foraging for Other Nutritive Plant Materials

Bees in several genera of Anthophorinae and Mellitidae collect oils from special floral glands, called elaiophors (Buchmann and Hurley 1978; Vogel 1974) that occur in plants belonging to at least five dicot and two monocot families. Oil-collecting bees have morphological specializations, such as modified setal combs on the tarsi and thickened scopal setae (Simpson et al. 1977). The oil is mixed with pollen, and possibly nectar, and used in larval provisions. Baker et al. (1973) suggested that stigmatic exudates serve as food for flower visitors. These substances are high in lipid content and may contain amino acids and other nutrients. However, they sometimes contain substances that are toxic to bees, such as galactose in tulip exudates (Barker and Lehner 1976). Abrol (1985) observed *A. florea* foraging on extra floral nectaries of peach located at the base of leaves.

11.20 Foraging in Relation Propolis

A. cerana is one of the Asian honeybee species that does not use propolis, whereas *A. florea* on the other hand deters enemies by using rings of propolis (like grease bands) to coat the branch from which its single-comb nest is suspended (Abrol 2009). However, *A. dorsata* was not observed collecting propolis. Different races of *A. mellifera* are known to collect propolis. Propolis is only collected when the temperature is above 18 °C. Each corbicula can carry about 10 mg of propolis. Because of its stickiness, propolis gathering is a slow business, it can take an hour to fill both baskets, and back at the hive, unloading can take another hour (Nicolson 2009).

11.21 Foraging in Relation to Biochemical Characters of Host Plants

Insects require appreciable amount of protein, carbohydrate, lipid and nitrogen for growth and development (Bertsch 1983, Murugan and George 1992). A direct correlation is expected to exist between the honeybee visits and biochemical nature of flower parts. Nathan et al. (1999) monitored the foraging activity of *A. florea* on *Ipomea staphylina* and *Eucalyptus tereticornis* flowers and found that in both these plants the nectar secretion activity is minimal thus forcing the bees to make repeated

Table 11.3 *Apis florea* visit to *Ipomea staphylina* and *Eucalyptus tereticornis* during different times of the day. (Source: Nathan et al. 1999)

Time (IST) (Hours)	Visits/30 min/inflorescence	
	<i>Ipomea staphylina</i>	<i>E. tereticornis</i>
0630–0700	5	8
0700–0730	5	8.4
0730–0800	6	8.9
0830–0900	6.2	9
900–0930	6	7.5
1200–1230	–	1.3
1300–1330	–	1
1600–1630	1.4	3
1630–1700	1.0	2

IST Indian standard time

visits to a large number of flowers to satisfy their nutritional demands. Interestingly, they found that *A. florea* stayed for longer time in the flowers of *I. staphylina* than *E. tereticornis*. The increased rate of visit by *A. florea* to the flowers of *E. tereticornis* compared to *I. staphylina* was attributed to higher amount of protein, carbohydrate and optimum level of lipid and nitrogen in *E. tereticornis*. Furthermore, *A. florea* preferred to visit *E. tereticornis* than *I. staphylina* since *E. tereticornis* had higher flower density. Percentage of *A. florea* population was highest in the months of November, December, January and February. Foraging population was greater in the morning (6–10 h) with moderate peak at 9 h, decreased gradually in the afternoon (12–14 h) and suddenly dropped up to 18 h under Coimbatore conditions (Western Ghats 10° 58' N, 82° 18' E). They further observed that *A. florea* confined their visits to *I. staphylina* and *E. tereticornis* and did not make inter-plant movements (Table 11.3 and 11.4).

Table 11.4 Nutritional components from flower parts of *Ipomea staphylina* and *Eucalyptus tereticornis*. (Source: Nathan et al. 1999)

Biochemical parameters	Anthers	Pollen	Stigma	Petals
Protein (mg/g)				
<i>Ipomea staphylina</i>	92.34 + 6.85	121.08 + 8.96	112.40 + 7.31	89.93 + 6.92
<i>E. tereticornis</i>	95.58 + 7.31	120.60 + 8.96	140.21 + 10.38	93.68 + 8.94
Carbohydrate (mg/g)				
<i>Ipomea staphylina</i>	98.42 + 8.53	111.02 + 9.18	87.01 + 7.48	132.48 + 11.56
<i>E. tereticornis</i>	101.31 + 8.87	115.65 + 8.34	91.38 + 7.34	143.56 + 9.26
Lipid (mg/g)				
<i>Ipomea staphylina</i>	25.05 + 2.50	36.81 + 2.89	33.18 + 2.75	21.08 + 2.83
<i>E. tereticornis</i>	31.69 + 2.47	43.86 + 3.63	39.15 + 2.98	27.01 + 2.10
Nitrogen (%)				
<i>Ipomea staphylina</i>	1.29 + 0.078	1.08 + 0.013	1.34 + 0.021	2.18 + 0.065
<i>E. tereticornis</i>	1.38 + 0.07	1.15 + 0.07	1.49 + 0.06	2.58 + 0.08
Water content (%)				
<i>Ipomea staphylina</i>	42.19 + 2.81	38.03 + 2.73	55.05 + 3.92	75.02 + 4.68
<i>E. tereticornis</i>	45.36 + 2.94	36.91 + 2.11	57.90 + 3.95	69.01 + 4.80
Mean + S.E. of 5 observations				

S.E. standard error

11.22 Influence of Honeybees on Other Bee Species

The introduced honeybee species have been reported (Pearson 1933) to influence native bees in a profound manner. Several authors have since presented experimental evidence of competition between honeybees and wild bees for flower resources. Roubik (1978) reported that the introduced hives of Africanized honeybees near patches of various flower species in French Guiana appeared to displace stingless bees from some of the flower species. Displacement and even aggression between honeybees at artificial nectar sources has been reported. *A. mellifera* aggressively displaced *A. cerana* from artificial sugar feeders in Japan (Sakagami 1959). Abrol (2009) reported that introduction of *A. mellifera ligustica* have severely marginalized the indigenous honeybee *A. cerana* in India. Interactions suggesting competition between honeybees and wild bees in more natural situations have also been reported. Wratt (1968) found that the number of *Bombus ruderatus* foragers carrying pollen on plots of red clover in New Zealand decreased with increasing temperature, apparently due to competition by *Apis*. Taken together, these studies provide strong evidence that honeybees do influence the foraging patterns of native bees by competition at resource sites.

11.23 Nectar Robbing

Nectar robbing in cauliflower bloom by many honeybee foragers has been found in Solan, India by Kumar et al. (1994). Side foraging of cauliflower nectar by *A. cerana* and *A. mellifera* and of Okra nectar by *A. cerana* has been reported by Kapoor and Dhaliwal (1989) and Mishra et al. (1987), respectively. Kapil and Kumar (1975) reported that few foragers of *A. dorsata* collected nectar from *B. juncea* flowers without actually entering the flowers. Sharma et al. (2001) found that all the bees *A. florea*, *A. dorsata*, *A. cerana* and *A. mellifera* foraged as top workers on flowers of *B. campestris* var. *sarson*, *A. cepa*, *D. carota*, *T. alexandrinum* and *Helianthus annuus*, except that *A. florea* foraged as side worker on *Brassica* flowers.

11.24 Communication and Orientation in Honeybees

Communication in *Apis* species was compared by Lindauer (1957). He reported that the communication pattern in *A. cerana indica*, *A. dorsata* and *A. florea* was similar to *A. mellifera*, with slight differences. *A. cerana* could communicate when the food source was at distance of 2 m or more, *A. dorsata* at 4 m and *A. florea* at 5 m, sickle dance was performed when the distance was less than these. *A. cerana indica* and *A. dorsata* communicated the direction in wag-tail dance on a vertical comb surface but *A. florea* performed the dance on the top of the horizontal surface. In all the *Apis* species the communication tempo was correlated with the distance. Atwal and

Goyal (1969) and Goyal and Atwal (?) confirmed the finding of Lindauer. They found that *A. cerana indica* performed a round dance up to 0.3–0.7 m foraging distance, workers moved clockwise for 2–6 times, then anticlockwise several times. But *A. mellifera* performed a round dance for 0.5–0.27 m distance food source; workers alternated single clockwise and anticlockwise circles with increase in distance, the dance changed to sickle dance (8 m for *A. cerana indica* and 38 m for *A. mellifera*). They have also reported simple conditioning in dish feeding tests, and trained bees were found to escort the new ones to feeding stations. Dixit (1956) in his studies on visual activity found that bees preferred the black figures to grey background and *A. cerana indica* preferred more subdivided figures. He further reported that *A. florea* also behaved similarly.

Kirchner et al. (1996) studied the dance language of the giant honeybee *A. laboriosa*, which is exclusively diurnal and closely related to *A. dorsata* and found that this species do not contain any acoustic signals, indicating that acoustic signalling of the location of food sources is indeed restricted to species which need to dance under low light intensities. *A. laboriosa* has never been found to be active at night and nocturnal activity would seem to be quite unlikely because of low temperatures at night time. The dance language of this species is therefore especially interesting as *A. laboriosa* dances silently, whereas *A. dorsata* produces dance sounds. The facts that *A. mellifera* and *A. cerana* use sound signals that are quite similar to those of *A. dorsata*

11.25 Effect of Pathogens and Diseases on Foraging Activity of Bees

Anderson and Giacon (1992) reported that honeybee, *A. mellifera* colonies fed on preparations of 50 % of sucrose containing sac brood virus particle and *Nosema Apis* Zander spores collected significantly less pollen than colonies fed only on the sucrose solution. Bailey and Fernando (1972) found that worker bees infected with sac brood virus have a foraging behaviour different from non-infected bees in that they collect mainly nectar rather than pollen. Evidently, infection of either sac brood or *Nosema* in honeybees may reduce their pollinating efficiency to pollinate plant crops especially those plants that flower during spring or early summer or that produce only pollen, such as kiwifruit, *Actinida deliciosa*. Abrol (1995a) found that *A. cerana* and *A. mellifera* infested with ectoparasitic mites had reduced pollen collection activity as compared to healthy ones.

11.26 Foraging in Relation to Body Size

Abrol (2006b) studied the foraging behaviour of honeybee *A. florea* F. on carrot (*D. carota*) flowers. Of all these insects, the dwarf honeybee *A. florea* F. was the most predominant and comprised more than 94 % of the total flower visiting insects.

This may be due to the fact that *A. florea* had much suitability of its tongue length to the corolla length of carrot bloom. *A. florea* while collecting nectar or pollen lay across the umbel pollinating large number of flowers. This behaviour of *A. florea* makes it most efficient pollinator of carrot blossoms. They are probably less selective than other *Apis* species and their small size enables them to forage successfully on many plant species. Rewarding system developed by the flowers enable pollinators to discriminate between the closely related plant species or ecotypes. This has resulted in a co-partnership between the flowers and their pollen vectors. Co-evolution has brought a close correlation between pollinator needs and floral energy expenditures (Heinrich 1975a).

11.26.1 Foraging in Relation to Pheromones

There are several pheromones to attract bees for the crops that need cross pollination (Free 1962, 1968, 1981a, b, 1982, 1987; Free et al. 1989; Waller 1970; Woyke 1981, Woyke et al. 2001a, b; Ohe and Praagh 1983; Currie et al. 1992) and to repel them to save from hazardous effects of pesticides (Atkins Jr. et al. 1975a, 1975b; Attri and Singh 1978; David and Somasundaran 1985). Use of synthetic queen pheromones to stimulate foraging (especially pollen collection) and Nasonov pheromone whose two components (citral and geraniol) have been reported to increase honeybee foraging in onion (Woyke 1981) and yield of apple (Ohe and Praagh 1983) hold a good promise. Use of synthetic alarm pheromones, a few minutes before insecticide application may help in repelling them from the crop thereby reducing bee loss, and some other pheromones need further refinement. Repellents sprayed on the target crops reduce the bee forage and induce them to forage on the less attractive target crops, e.g. carbolic acid, acetic acid, propionic anhydride, benzaldehyde, calcium chloride. The mandibular gland pheromones can also be used. Synthetic pheromones ((E)- and (Z)-citral, nerol, geraniol, (E, E)-farnesol and (E) 9-oxo-2 decanoic acid) have been reported to induce clustering of *A. cerana*. Similarly, pheromones influencing the clustering activity of *A. mellifera* such as 9-oxo-2decanoic acid (queen bee factor), (E) and (Z)-citral, nerol, geraniol and (E, E)-farnesol (worker bee factor) were found to induce clustering in *A. dorsata*. *A. florea* foragers were observed to leave pheromone at the source of forage that was attractive to other foragers (Butler 1954). However, they were not seen to expose their Nasonov gland when foraging on flowers, or at dishes of sugar syrup (Free and Williams 1979). A similar forage-marking pheromone has not been demonstrated for *A. mellifera* (Dutton and Simpson 1977). This pheromone is non-specific, and is probably always deposited when a bee forages, irrespective of the attractiveness of the food source and whether it has an odour; it is probably a more primitive form of communication than the Nasonov pheromone.

Neira et al. (1996) evaluated the effect of two attractants on the pollination efficiency of honeybees (*A. mellifera* L.) on pear trees and observed that trees cv. 'Red Bartlett' treated with Beescent (5 l/ha) or Beeline (5 kg/ha) attracted significantly higher number of bees compared to control for 3 days. Singh and Sinha (1996)

studied the effect of Bee-Q on honeybee visit and seed yield of hybrid sunflower and found that there was no increase in the number of honeybee visits or in percentage seed set, number of filled seeds/head or weight of 1,000 seeds compared to unsprayed plots. Viraktamath (1999) also studied the influence of bee attractants on the visitation of *A. dorsata*, *A. mellifera* and *A. cerana* on yield parameters of *Sesamum indicum* and found that flowers sprayed with Bee-Q or Bee-Here or 10 % sugar solution had significantly more bee visits than on the untreated control and mean number of pods/plant and number of seeds/pod were significantly higher than on the control. Bhat and Sudharshan (1999) found that when flowering plants of cardamom (*Elettaria cardamomum* Maton) were sprayed with Bee-Q, the number of bee visits doubled (104.8 % increase), fruit set increased by 13 % compared with unsprayed control plants, and recovery of seed also increased slightly. Patil et al. (2000) studied the effect of Bee-Q and Bee-here on pollinators and yield of *Sesamum* and reported that spraying sesame plants with these bee attractants was effective in attracting higher numbers of pollinators (*Apis* spp.), and resulted in higher yields, germination and shoot length than on caged plants. Sanjivan et al. (2000) evaluated four attractants viz. sugar syrup containing scent of sunflower, spraying sugar syrup on the flowers, spraying trionic acid and spraying Bee-Q for increasing the attractiveness of sunflower to honeybees for pollination and found that all of them increased bee visits and gave maximum seed set, 1,000 seed weight, yield and oil content. Several compounds have been reported as repellents. Naik et al. (2003) reported *Fagara budrunga* fruit extract as an attractant for *A. cerana*. Naik et al. (2002) found 2-heptanone as a repellent for *A. florea* and Ethylthio cyclopentane for *A. cerana* (Naik et al. 1999).

11.27 Foraging in Relation Cellphone Radiations

Sharma and Kumar (2010) has reported that use of electronic gadgets has led to electropollution of the environment adversely affecting behaviour and biology of honeybee, *A. mellifera*. They found that the behaviour of foragers exposed to average radiofrequency (RF) of 8.549 $\mu\text{W}/\text{cm}^2$ (56.8 V/m, electric field) for 15 min, twice a day during the period of peak bee activity (1100 and 1500 hours) was negatively influenced by the exposure, in terms of significant decline in flight activity, returning ability of bees to their hives and pollen foraging efficiency (Table 11.5). The orientation behaviour is affected by electrosmog since these insects have magnetite in their bodies which helps them in navigation. Since other honeybee species *A. florea*, *A. dorsata*, *A. laboriosa* and *A. cerana* co-exist sympatrically, such a decline due to electromagnetic radiation is very much expected and needs exploration.

11.28 Foraging in Relation Water

A honeybee colony adaptively controls the collection of water by its foragers, increasing it when high temperatures necessitate evaporative cooling inside the hive and decreasing it when the danger of overheating passes. Honeybee colonies collect

Table 11.5 Changes in foraging behaviour of *Apis mellifera* exposed to cellphone radiations. (Source: Sharma and Kumar 2010)

Parameter	Control (mean + SD)	Treated (15 min exposure) (mean + SD)
Flight activity (No. of worker bees leaving the hive entrance/min)		
Before exposure	35.9 + 13 (12–61)	34.1 + 10 (18–48)
During exposure	37.2 + 12 (12–72)	22.8 + 6 (13–34)
Returning ability (No. of worker bees returning to the hive/min)		
Before exposure	39.6 + 13 (12–61)	36.4 + 11 (21–58)
During exposure	41.3 + 11 (14–78)	28.3 + 8 (16–48)
Pollen foraging efficiency (No. of worker bees returning with pollen loads/min)		
Before exposure	7.0 + 2 (4–9)	6.3 + 2 (4–10)
During exposure	7.2 + 2 (4–11)	4.6 + 2 (2–7)

SD standard deviation



Fig. 11.1 *Apis dorsata*, *Apis florea* and *Apis cerana* collecting water

water for two reasons, related to different types of weather: for cooling of the brood area by evaporation on hot days, and for feeding the larval brood when foraging is limited on cool days (Lindauer 1955; Seeley 1995). The classic studies of Lindauer showed how bees regulate the hive temperature in hot conditions (Lindauer 1955). Water is collected by water foragers, then distributed around the hive and in cells containing eggs and larvae; fanning accelerates its evaporation, as does regurgitation and evaporation on the tongue (Lindauer 1955). Visscher and colleagues measured mean water loads of 44 mg in honeybees collecting water under desert conditions (Visscher et al. 1996). All the honeybee species *Apis florea*, *A. dorsata*, *A. cerana* and *A. mellifera* collect water when temperature exceeds 38 °C (Abrol 2009; Fig. 11.1). Apart from environmental factors, the tendency of honeybee foragers to collect water, nectar or pollen has a genetic component (Hunt et al. 1995). Workers with the lowest sucrose response thresholds, i.e. those able to distinguish low sucrose concentrations from water in proboscis extension response tests, become water foragers (Pankiw and Page 2000).

11.29 Water Foraging in Relation to Salts and Nutrients

Abrol et al. (2012) found that *A. dorsata* collects water from animal wastes. Foraging of bees on animal wastes may be due to their need for water, salts and amino acids, which are available in animal wastes including urine.

11.29.1 Control of Foraging

A major crop pollination goal is to control foraging bees and get them to more effectively visit and pollinate crops; conversely, we would like to repel them from areas where there is danger from insecticides or where they endanger people. Work with other insects, both social and non-social, indicates that this is possible by chemical and physical means. There is considerable evidence that different plant species produce varying attractant compounds associated with their nectar and pollen. Bees are highly attracted to the scent of recently extracted honeycomb and to the scent of honey being extracted or heated. Obviously, chemical scents of certain flowers and to some extent scents incorporated in the collected honey are attractive to bees or associated with available food. Some pollens also contain chemical compounds that stimulate collection response in bees. Isolation and identification of these bee-attractive compounds and the application of the attractant to plant areas or altering attractants through plant breeding are an area of research of potential importance to crop pollination. Research should not be confined to chemicals alone, but should be shared equally with various physical factors that can possibly attract or repel bees. In other entomological fields, research on physical methods of controlling insects is receiving intensive investigation. Different insects respond in differing ways; they are attracted to certain light wavelengths and repelled by others.

11.30 Conclusions

Bees are excellent test animals for optimal foraging theories, because energy gained from nectar can be accurately measured. Most energetics research is on bumblebees and honeybees, primarily because of their high energy requirements and because they are easily followed while foraging. More work is needed on the energetics of foraging in other taxa of bees and on the importance of factors other than energy, such as nutritive values of different pollens, in determining bee foraging patterns. No bee species is an absolute generalist forager. All bees specialize, to some extent, through innate or learned preferences for particular plant taxa, foraging times, spatial distributions of flowers, floral structures, or floral products. Most studies of competition for floral resources among bee species, especially between honeybees and wild bees, have been inconclusive because competition is difficult to prove in the field. Future studies should include manipulation of forager and resource levels and behavioral observations of the interactions between bees on flowers.

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Chapter 12

Floral Resources

12.1 Introduction

The natural diet of the bees consists of carbohydrates, proteins, vitamins, salts, etc. The nectar of the flowers, which the bees collect and convert into honey, is the source of carbohydrates and vitamins. Pollen, the yellow powder in the flowers, is the main source of protein and it is mixed with honey before it is fed to the larvae. The winged bees, when they are young, also feed on this mixture. Soon they become strong and secrete the royal jelly, which forms the food of the queen and of the larvae in the early stages of growth. These young bees thus act as nurse bees, but as they grow older they take up the field duties and feed on honey only. In a hive, the nurse bees are often seen going from one cell to another, feeding the young larvae. For brood rearing, it is important that there are plenty of flowers in nature as the source of pollen and honey. For the growth of one larvae of honeybees into an adult bee, one cell full of honey and one cell of pollen is required. In other words, two frames of honey and pollen are required by the bees to raise one frame of brood. Bees can also use the sugar syrup as food (sugar dissolved in an equal quantity of water). Sugar is offered to supplement honey resources or in the extreme case to save the weak colonies from starvation. Early in the spring, when the flowers are not in abundance, bees can be stimulated to start brood rearing, and it should synchronise with the main honey flow so that the bees can take best advantage of it. Under proper management, not more than 1 or 2 kg of sugar are needed to be used in a year per colony. A colony of normal size should have at least six to eight pounds of honey (two or three frames) in reserve. When the stores fall below this level, bees should be fed artificially. If sugar is given as a winter reserve, the syrup should be thick, prepared by mixing two parts of sugar with one part of warm water.

Collection of food in honeybees is a social enterprise and more than 10,000 of foragers may be engaged together in collecting nectar and pollen. The foraging is designed in a way to achieve high efficiency. The foragers sacrifice their individual foraging efficiency for colony efficiency. When they discover a rich source of food, they communicate the information through various types of dances to their hive mates. High foraging efficiency is achieved as a result of sharing information about location of rich food sources. Four important resources include nectar, pollen, water,

and resin. Nectar and pollen are diet, water is used for evaporative cooling of the nest in hot days, and resin is used for plugging the unwanted openings.

Colonies managed for honey production rear 150,000–200,000 bees annually and consume 15–30 kg of pollen and up to 80 kg of honey. The number of trips required to pool these materials could be quite astonishing. To collect 20 kg of pollen, approximately 1–3 million foraging trips are required. Each trip on an average measures 4–5 km of distance. Likewise, to collect 60 kg of honey, 3 million foraging trips are required. In brief, each colony can be thought of as an organism which weighs 1–5 kg (biomass of bees 7,700 bees/kg), rears, 150,000 bees, and consumes 20 kg of pollen and 60 kg of honey every year. To collect this food, several million foraging trips are required and foragers fly about 20 million kilometers.

12.2 Bee Flora and Its Importance in Beekeeping

The plants that yield both pollen and nectar are called bee pasturage. However, honey bees gather nectar, pollen, propolis and water as their food. Nectar is a sweet secretion from the floral and extra floral nectarines of blossoms. It is the basic raw product of honey. It consists of dissolved sugar, i.e., sucrose, glucose, and fructose. The plants that produce only nectar are called bee forage. Pollen is a highly proteinaceous food for bees. The plants that produce only pollen are called pollen plants. Pollen, being completely independent plant cell, contains the substances which make up a living cell, and is therefore very good bee food. It provides the bees all their requirements of aminoacids, vitamins, and minerals.

The amount of pollen collected by a colony depends upon the colony's immediate requirements. There is a correlation between amount of brood present in a colony and the amount of pollen collected. A colony increases the number of bees collecting pollen, when the colony finds reduced amount of pollen arriving into the brood. Propolis is a resinous substance which is gathered mainly from buds and bark of trees like alder, poplar, horse, chestnut, and wounds of woody plants. It is used for sealing up cracks in the hive or nest and reducing the size of the entrance. Water is required inside the hive to regulate the temperature and to dilute stores of honey. Much water is collected in early spring and hot summer before the supply of nectar is readily available. The colony survives on its stored honey during the hot summer weather.

Honeybees are entirely dependent upon flowering plants for their food requirement. This means that if there are no flowers in any season, honeybees would not get their food. One of the major problems in beekeeping is the presence of floral dearth periods, which result in dwindling and desertion of the bee colonies. Every locality has floral dearth periods of long or short durations. Beekeepers have to ensure regular supply of food to the bee colonies to run the industry efficiently.

Nectar is the sweet liquid, which comes from floral and extra floral resources, and is the raw material for honey. Pollen is highly proteinaceous food for bees. The plants that yield both these substances are collectively called as "bee pasturage". The period when good number of plants providing nectar and pollen are available to bees, it is called as "honey flow period". If the nectar yield is copious from a good number

of plants of a particular species, it is called as “major honey flow period”. When the amount of nectar to be collected is small, it is called as “minor honey flow period”. The day when there is no honey flow, the period is called as “dearth period”. Since nectar and pollen plants are basic requirements for beekeeping and honey production, their knowledge is essential for beekeepers. Bee forage plants may be fruits, vegetables, oil-seeds, ornaments, crops, herbs, shrubs, bushes, forest and avenue trees and weeds in field. Efficient beekeeping means managing honeybee colonies in such a way as to obtain maximum colony population to coincide with the major honey flow in an area and to utilise the population for honey production and pollination. Evidently, the first step to start beekeeping in an area requires preparation of floral calendars to determine when honey flow occurs.

12.3 Floral Calendars

A floral calendar for beekeeping is a time-table that indicates to the beekeeper the approximate date and duration of the blossoming periods of the important honey and pollen plants in his area. Beekeeping in any specific area cannot develop without an understanding of the calendar, and for migratory beekeeping, special calendars for the different foraging zones along the migration route are required. Preparation of a floral calendar for any specific area requires complete observation of the seasonal changes in the vegetation patterns and/or agro-ecosystems of the area, the foraging behaviour of the bees, and the manner in which the honeybee colonies interact with their floral environment. The preparation of an accurate, detailed calendar will therefore often require several years of repeated recording and refinement of the information obtained.

12.4 To Determine When the Honey Flow Occurs

1. Make seasonal survey to identify the nectar and pollen yielding plants.
2. Make survey to record the flowering period of these plants.
3. Determine whether the plants are visited by honeybees for nectar, pollen or both
4. Determine whether bees could collect surplus honey from some abundant crops.
5. Determine what are the nectar-secreting flowering plants besides the major crops of the area?
6. How long a dearth period, if any, lasts?
7. Examine weather records data and altitudinal variations.

The steps required are as given below:

1. Place several strong honeybee colonies in the area, inspecting the hives regularly and observing changes in the amount of food stored within the hive to determine whether it is depleted, stable or increasing. Any food gains or losses can be monitored accurately by weighing the hives.

2. Within the flight range, record the species of plants that the bees visit. Determine whether the plants are visited for nectar or for pollen. Pollen-foragers will have pollen pellets attached to their hind legs. To determine whether the bees visit flowers for nectar, squeeze the abdomen of individual bees to obtain a drop of regurgitated nectar, tasting it for sweetness or measuring the nectar concentration with a hand refractometer.
3. Study the frequency with which the bees visit each flower species, in relation to changes in the level of the colonies' food stores. If there is a continuous increase in food stores, in direct response to the availability of the plants visited, the plants are good forage sources. When the food stores remain stable, the plants can be depended upon to meet the colonies' daily food requirements, but they cannot be classified as major honey sources.
4. Carefully records all the changes in the blossoming of the plants visited. When the colonies begin to lose weight, the flowering season is finished for all practical purposes.
5. Once all the data on forage species have been assembled and repeatedly verified, they should be judged as they relate to the actual performance of the honeybee colonies. Make floral calendar, in the form of a circular chart, indicating the periods of availability of major nectar and pollen sources.

The suitability of beekeeping in a locality will depend upon answers to these questions. If nectar secreting plants are available in a large number, that is there are one or two major honey flow periods with minor honey flow periods during other parts of the year and the dearth period of not long duration, then beekeeping can be successful in that area. During the honey flow period, a good colony of honeybees may collect 1–2 kg of honey per day. It should be kept in mind that a few fruit trees or flowering plants or a vegetable farm or garden cannot sustain any number of bee colonies leaving alone surplus honey is hoped for, they must be able to take advantage of many across honey yielding pasturage.

April and May are usually considered to be the period of nectar flow. After honey flow, there comes the hot months of June and July when most of the colonies stop brood rearing in the lower hills and in the plains. The bees, which had put in hard work in collecting resources, die of old age and strength of the colony depletes. Some bees are also lost in their effort to keep the hives well ventilated and cool. The monsoon season starts in the first week of July and because of the heavy rains bees generally stay inside. Some of those, which go out for field duties, are lost in the heavy downpour and the strength of the colonies depletes further. This is a very difficult period for the bees.

The honey flow period, dearth periods, etc., varies from one location to another and with altitudes. The flowering periods of even the same plant species vary in different geographical regions and agroclimatic zones. For instance, in India, in the lower hills and valley areas of Himachal Pradesh, the season for activities of bees starts right from the advent of the winter when sarson flowers are available in the fields. Then fruit crops provide flowers in the second week of February. They are followed by the flowering of Eucalyptus, Shisham (*Dalbergia sissoo*), Tun (*Cedrella toona*), and their flowers continue upto the end of April. After a gap of about 1 week,

the soapnut (*Sapindus* sp.) starts blooming which provides the main honey flow. It continues upto the end of May and surplus honey is extracted in the end of May or the first week of June. Under Punjab (India) conditions, surplus honey is stored from Eucalyptus, shisham, citrus, stone fruits, litchi, and barseem flowers in March, April, and May. Then follows the dearth period up to September and during this period the bees visit a number of wild flowers and the blossoms of maize in the end of July. In the oil-seed and cotton growing areas bees also store surplus honey from toria, cotton, arhar, etc., in September and October. In the higher altitudes of Himachal Pradesh (India), winter is much colder and the bees are very much restricted in their activities. The stone fruits including pear, almond, plum, cherry, apple, apricot, etc., blossom in the spring and the bees are stimulated to rear their broods. In the beginning of May, barberry flowers provide minor honey flow periods followed by soapnut in the end of the June. Early in August, maize inflorescences provide pollen for brood rearing to bees. The colony strength increases. At the end of August, *Plectranthus* blossoms appear which continue up to the end of October. This provides the main honey flow season. Extraction is done towards the end of the October, leaving sufficient honey reserves for the bees to tide over winter. In Kashmir (India), the activity is instigated with the appearance of early blossoms from *Salix*, *Narcissus*, *Brassica*, *Pyrus*, *Prunus*, etc. in Feb–March. During April and June blossoms of *Iris*, *Robinia*, *Corylis*, *Castanea*, *Aesculus*, *Alianthus*, *Rose*, etc., provide the requirements at the peak of brood rearing activity and occasionally also lead to a spring honey crop (Table 12.1).

While fruit blossoms, mustards and other early flowering herbs greatly help in encouraging brood rearing activity and in building up some reserves of nectar and pollen. Vegetable blossoms and other flowering annuals and trees, which appear during May and June, perform valuable sources. July and August are the dearth periods. During August and September, colonies are migrated to higher altitudes where pollen and nectar sources such as *Zea*, *Zinnia*, *Thyme*, *Mentha*, *Cyanoglossum*, *Balsam* etc. play an important role in gearing up the bee activity. Among the various shrubs providing nectar or pollen or both, *Plectranthus rugosus* Wall, which flowers from mid-August to mid-October, provides for a major part of the honey flow in autumn, because of its abundance on many hillocks in Kashmir. Saffron (*Crocus*) blossoms during late October and mid-November, if availed, are of immense value to bees for brood rearing even in late fall.

In Jammu, India, April provides the major honey flow period, June–July–August are dearth periods (Table 12.2). During August and September colonies are migrated to higher altitudes to utilise *Plectranthus rugosus*. October onwards rapeseed and mustard crops are available up to Feb, followed by fruit blossoms etc. J&K state has varied climates, habitats, which support varied type of vegetation and cultivated crops. Broadly it may be divided into: (1) Subtropical zone, (2) Intermediate zone, (3) Temperate zone. The vegetation and blooming timing vary from one region to another. Similarly, floral calendars of beekeeping plants are available in different states. However, a particular crop may be good bee plant in one locality but can be quite otherwise in another locality. Honey production from a crop in the same area may vary from year to year.

Table 12.1 Plant species of Kashmir region

Scientific name	Family	Common name	Flowering time	If pollen or nectar yield	Bee species
(1)	(2)	(3)	(4)	(5)	(6)
<i>Salix caprea</i>	Salicaceae	Pussy willow	Feb–March	N + P	<i>Apis cerana</i>
<i>Gagea Kashmiriana</i>	Liliaceae		Feb–March	P	<i>Apis cerana</i>
<i>Colchium Leutem</i>	Iridaceae	Colchicum	Feb–March	P	<i>Apis cerana</i>
<i>Narcissus poeticus</i>	Amaryllidaceae	Daffodil	Feb–March	N + P	<i>Apis cerana</i>
<i>Endymion non-scriptus</i>	Liliaceae	Hyacinth	Feb–March	N + P	<i>Apis cerana</i>
<i>Brassica juncea</i>	Cruciferae	Mustard	March–April	N + P	<i>Apis cerana</i>
<i>Prunus amygdalus</i>	Rosaceae	Almond	March–April	N + P	<i>Apis cerana</i>
<i>Prunus avium</i>	Rosaceae	Cherry	March–April	N + P	<i>Apis cerana</i>
<i>Fritellaria imperialis</i>	Liliaceae	Frietelliria	March–April	N + P	<i>Apis cerana</i>
<i>Viola odorata</i>	Violaceae	Sweet Voice	March–April	N + P	<i>Apis cerana</i>
<i>Plantago media</i>	Plantaginaceae	Hoary Plantain	March–May	P	<i>Apis cerana</i>
<i>Vitis parvifolia</i>	Vitaceae	Grape	March–May	N + P	<i>Apis cerana</i>
<i>Taraxacum officinale</i>	Compositae	Dandelion	March–June	N + P	<i>Apis cerana</i>
<i>Primula spp.</i>	Primulaceae	Primula	March–June	P	<i>Apis cerana</i>
<i>Prunus</i>	Rosaceae	Apricot	April–May	N + P	<i>Apis cerana</i>
<i>Prunus persica</i>	Rosaceae	Plum	April–May	N + P	<i>Apis cerana</i>
<i>Pyrus malus</i>	Rosaceae	Apple	April–May	N + P	<i>Apis cerana</i>
<i>Pyrus persica</i>	Rosaceae		April–May	N + P	<i>Apis cerana</i>
<i>Juglans regia</i>	Juglandaceae	Walnut	April–May	N + P	<i>Apis cerana</i>
<i>Morus alba</i>	Moraceae	Mulberry	April–May	P	<i>Apis cerana</i>
<i>Raphanus sativus</i>	Cruciferae	Radish	April–May	N + P	<i>Apis cerana</i>
<i>Bellis perennis</i>	Composite	Daisy	April–May	N + P	<i>Apis cerana</i>
<i>Daphne oleides</i>	Thymeliaceae		April–May	N	<i>Apis cerana</i>
<i>Podyphyllum emodi</i>	Berberidaceae		April–May	N + P	<i>Apis cerana</i>
<i>Rosa macrophylla</i>	Rosaceae	Rose	April–June	P	<i>Apis cerana</i>
<i>Cucumis melo</i>	Cucurbitaceae	Musk melon	April–June	N + P	<i>Apis cerana</i>
<i>Cucumis moschata</i>	Cucurbitaceae	Pumpkin	April–June	N + P	<i>Apis cerana</i>
<i>Cucumis sativus</i>	Cucurbitaceae	Cucumber	April–June	N + P	<i>Apis cerana</i>
<i>Rosa sp.</i>	Rosaceae	Wild rose	April–June	P	<i>Apis cerana</i>
<i>Fargaria spp.</i>	Rosaceae	Strawberry	April–June	N + P	<i>Apis cerana</i>
<i>Iris spp.</i>	Iridaceae	Iris	April–June	N + P	<i>Apis cerana</i>
<i>Anemone nemorosa</i>	Ranunculaceae	Wood Anemone	April–June	N + P	<i>Apis cerana</i>
<i>Runex spp.</i>	Polygonaceae	Dock	April–June	N	<i>Apis cerana</i>
<i>Caltha palustris</i>	Ranunculaceae	Marsh marigold	April–July	N + P	<i>Apis cerana</i>
<i>Robinia pseudoacacia</i>	Leguminosae	Robinia	Late Apr.–Mar	N + P	<i>Apis cerana</i>
<i>Trifolium repens</i>	Leguminosae	White clover	April–August	N	<i>Apis cerana</i>
<i>Pyrus communis</i>	Rosaceae	Pear	Early May	N + P	<i>Apis cerana</i>
<i>Cydonia oblonga</i>	Rosaceae	Quince	Mid May	N + P	<i>Apis cerana</i>
<i>Citrullus vulgaris</i>	Cucurbitaceae	Water melon	May–June	N + P	<i>Apis cerana</i>

Table 12.1 (continued)

Scientific name	Family	Common name	Flowering time	If pollen or nectar yield	Bee species
(1)	(2)	(3)	(4)	(5)	(6)
<i>Allium cepa</i>	Liliaceae	Onion	May–June	N + P	<i>Apis cerana</i>
<i>Allium sativum</i>	Liliaceae	Garlic	May–June	N + P	<i>Apis cerana</i>
<i>Capsicum frutescens</i>	Solanaceae	Chillies	May–June	P	<i>Apis cerana</i>
<i>Brassica oleracea</i> var. <i>capitata</i>	Cucurbitaceae	Cabbage	May–June	N + P	<i>Apis cerana</i>
<i>Tilia</i> spp.	Tiliaceae	Bass wood	May–June	N + P	<i>Apis cerana</i>
<i>Viburnum nervosum</i>	Caprifoliaceae	Viburnum	May–June	N + P	<i>Apis cerana</i>
<i>Rubus nivens</i>	Rosaceae	Raspberry	May–June	N + P	<i>Apis cerana</i>
<i>Peganum hermala</i>	Rutaceae	Rue	May–June	N + P	<i>Apis cerana</i>
<i>Strobilanthus alatus</i>	Acanthaceae		May–June	N + P	<i>Apis cerana</i>
<i>Delphinium denudatum</i>	Ranunculaceae	Denuded lark spur	May–June	N + P	<i>Apis cerana</i>
<i>Ranunculus bulbosus</i>	Ranunculaceae	Buttercup	May–June	N + P	<i>Apis cerana</i>
<i>Marrubium vulgare</i>	Labiatae	Hore Hound	May–June	N	<i>Apis cerana</i>
<i>Nepeta</i> spp.	Labiatae	Nepetae	May–June	N + P	<i>Apis cerana</i>
<i>Ailanthus</i> sp.	Simaroubaceae		Late May–June	N	<i>Apis cerana</i>
<i>Punica granatum</i>	Punicaceae	Pomegranate	May–June	P	<i>Apis cerana</i>
<i>Phaseolus</i> spp.	Leguminosae	Beans	May–June	N	<i>Apis cerana</i>
<i>Portulaca grandiflora</i>	Portilacaceae	Sun Plant	May–June	P	<i>Apis cerana</i>
<i>Helianthus annuus</i>	Composite	Sun Flower	May–June	N + P	<i>Apis cerana</i>
<i>Althea officinalis</i>	Malavaceae	Holy hock	May–June	N + P	<i>Apis cerana</i>
<i>Artemissia absinthium</i>	Compositiae	Worm wood	May–June	P	<i>Apis cerana</i>
<i>Solanum melongena</i>	Solanaceae	Egg plant	May–June	N + P	<i>Apis cerana</i>
<i>Lycoperison esculentum</i>	Solanaceae	Tomato	May–June	N + P	<i>Apis cerana</i>
<i>Castanea dentata</i>	Fagaceae	Chest nut	June	N + P	<i>Apis cerana</i>
<i>Corylus</i> spp.	Betulaceae	Hazel nut	June	P	<i>Apis cerana</i>
<i>Aesculus hippocastanum</i>	Hippocastanac eae	Horse chestnut	June	N + P	<i>Apis cerana</i>
<i>Opuntia</i> spp.	Betulaceae	Cactus	June–July	N	<i>Apis cerana</i>
<i>Potentilla argyropylla</i>	Rosaceae	Silver weed	June–July	N	<i>Apis cerana</i>
<i>Polygonum amplexicaule</i>	Polygonaceae	Polygonum	June–July	N + P	<i>Apis cerana</i>
<i>Discorea deltoidea</i>	Dioscoreaceae		June–July	N + P	<i>Apis cerana</i>
<i>Stellaria Bulbosa</i>	Caryophyllaceae		June–July	N	<i>Apis cerana</i>
<i>Papaver roheas.</i>	Papaveraceae	Field poppy	June–July	P	<i>Apis cerana</i>
<i>Lonicera sempervirens</i>	Caprifoliaceae	Honey suckle	June–July	P (mostly)	<i>Apis cerana</i>

Table 12.1 (continued)

Scientific name	Family	Common name	Flowering time	If pollen or nectar yield	Bee species
(1)	(2)	(3)	(4)	(5)	(6)
<i>Rhododendron companulatum</i>	Ericaceae	Rhododendron	June–July	N + P	<i>Apis cerana</i>
<i>Trifolium medicago</i>	Leguminosae		June–July	N	<i>Apis cerana</i>
<i>Gladiolus spp.</i>	Irdacaceae	Gladiolle	June–July	N + P	<i>Apis cerana</i>
<i>Dispasacus inermis</i>	Dipsacaceae	Smooth headed Teasel	June–Aug	N	<i>Apis cerana</i>
<i>Berberis lycium</i>	Berberideaceae	Barbery	June–Aug	N + P	<i>Apis cerana</i>
<i>Rhus spp.</i>	Anacardiaceae	Rhus	June–Aug	N	<i>Apis cerana</i>
<i>Carduus nutans</i>	Composite	Thistle	June–Aug	N + P	<i>Apis cerana</i>
<i>Abelmoschus esculentus</i>	Malvaceae	Lady finger			<i>Apis cerana</i>
<i>Malva sylvestris</i>	Malvaceae	Mallow	June–Sept.	N + P	<i>Apis cerana</i>
<i>Cichroium intybus</i>	Composite	Chicory	June–Sept.	N + P	<i>Apis cerana</i>
<i>Datura stramonitum</i>	Solanaceae	Thron apple	June–Sept.	P 83	<i>Apis cerana</i>
<i>Mentha sylvestris</i>	Labiatae	Horse mint	June–Sept.	N	<i>Apis cerana</i>
<i>Humulus Lupulus</i>	Moraceae	Hop	July–Aug	P	<i>Apis cerana</i>
<i>Gossysium spp.</i>	Malavaceae	Cotton	July–Aug	N + P	<i>Apis cerana</i>
<i>Nelumbium speciosum</i>	Nymphaceae	Lotus	July–Aug	P	<i>Apis cerana</i>
<i>Sophora spp.</i>	Leguminosae	Wild spora	July–Aug	N + P	<i>Apis cerana</i>
<i>Salvia hians</i>	Labiatae	Salvia	July–Aug		<i>Apis cerana</i>
<i>Macrootomia Benthami</i>	Borgainaceae		July–Aug	N + P	<i>Apis cerana</i>
<i>Medicago sativa</i>	Leguminosae	Alfalfa	July–Aug	N + P	<i>Apis cerana</i>
<i>Zinna elagans</i>	Composite	Zinna	July–Sept	P	<i>Apis cerana</i>
<i>Zea mays</i>	Gramineae	Maize	July–Sept	P	<i>Apis cerana</i>
<i>Impatiens glandulifera</i>	Balsaminaceae	Touch me not (Balsam)	July–Sept	N + P	<i>Apis cerana</i>
<i>Melilotus officinalis</i>	Leguminoase	Common Mellilot	July–Sept	N	<i>Apis cerana</i>
<i>Aster spp.</i>	Composite	Aster	July–Sept	N + P	<i>Apis cerana</i>
<i>Cynoglossum slochiitatum</i>	Boraginaceae	Honds Tounge	July–Oct	N + P	<i>Apis cerana</i>
<i>Delphinium incanum</i>	Ranunculaceae	Hoary lark spur	August	N	<i>Apis cerana</i>
<i>Swerita spp.</i>	Gentianaceae	Sweritia	Aug–Sep	N	<i>Apis cerana</i>
<i>Fagopyrum esculentum mill</i>	Polygoaceae	Buckwheat	Aug–Oct	N + + P	<i>Apis cerana</i>
<i>Plectranthus rugosus</i>	Labiatae		Mid Aug–Oct	N + P	<i>Apis cerana</i>
<i>Crocus sativus</i>	Irdaceae	Saffron	Late Oct–Nov	N + P	<i>Apis cerana</i>
<i>Bauhinia spp.</i>	Leguminosae	Geranium tree	Dec–Jan	N + P	<i>Apis cerana</i>
<i>Euphorbia pulcherrima</i>	Euphorbiaceae	Poinsettia	Dec–Jan	N + P	<i>Apis cerana</i>
<i>Mangifera indica</i>	Anacardiaceae	Mango	Jan–Feb	N + P	<i>Apis cerana</i>
<i>Citrus spp.</i>	Rutaceae	Citrus plants	Jan–Feb	N + P	<i>Apis cerana</i>
<i>Cedrella toona Roxb.</i>	Meliaceae		April	N + P	<i>Apis cerana</i>
<i>Dalbergia toona Roxb</i>	Leguminosa		April	N + P	<i>Apis cerana</i>
<i>Cassia spp.</i>	Leguminosa	Cassia	March–May	N + P	<i>Apis cerana</i>
<i>Melia indica</i>	Meliaceae	Pride of India	April–May	N	<i>Apis cerana</i>

Table 12.2 Plant species of Jammu region

Family Plant species	Common name	Flowering period	Source	Bee species
Aizoaceae				
<i>Mesenbryanthemum</i>	Ice	Feb	NP	dmc
<i>Crystallimum</i>	Plant April			
Acanthaceae				
<i>Adhatoda vasica</i>	Brankad	March–May	N	dm
Amaranthaceae				
<i>Celosia argentia</i>	Ciryara	Aug–Sep	NP	dmc
Amaryllidaceae				
<i>Narcissus poeticus</i>	Daffodil	Feb–March	NP	d
Anacardiaceae				
<i>Mangifera indica</i>	Aam	Mar–April	NP	dfcm
<i>Lannea coromadelica</i>	Kamibal	Apr–June	P	dc
Apocynaceae				
<i>Carissa spinarum</i>	Garna	Apr–May	N	f
<i>Grewia microcus</i>	Phalsa	Apr–May	P	dc
<i>Nerium indicum</i>	Kaner	Apr–May	N	dm
<i>Thevetia Peruviana</i>	Pilikaner	Apr–Nov	N	dm
Balsamnaceae				
<i>Impatiens glandulifera</i>	Balsam	July–Sep	Np	dmc
Bignoniaceae				
<i>Delonix regia</i>	Gulmohar	Mar–May	NP	dm
<i>Jacarnda acutifolia</i>	Nili	Mar–May	NP	dmc
<i>Jacarnda acutifolia</i>	Gulmohar			
<i>Tecoma stans</i>	Tecoma	Feb–March	NP	dmc
Bombaceae				
<i>Salmaal malabarica</i>	Simbal	Feb–March	N	d
Cactaceae				
<i>Opuntia spp.</i>	Cactus	May–July	N	d
Caesalpinaceae				
<i>Bauhinia variegata</i>	Kanchar	Mar–April	NP	dfmi
<i>Cassia spp.</i>	Amaltas	May–June	NP	d
Caesalpinaceae				
<i>Bauhinia Varigata</i>	Kanchar	Mar–April	Np	dfmc
<i>Cassia Fistula</i>	Amaltas	May–June	NP	d
Combretaceae				
<i>Terminalla belerica</i>	Behda	June–July	N	dc
<i>Terminalla chebula</i>	Harar	June–July	N	dc
<i>Terminalla arjuna</i>	Arjun	May–June	N	d
Commelinaceae				
<i>Commelina bengalensis</i>	Shura	Aug–Sep	P	dmc
Compositae				
<i>Helianthus annuus</i>	Sunflower	May–Sept	Np	dmc
<i>Cosmos sulphuresus</i>	Cosmos	Apr–May	Np	dmc
<i>Cersium sp.</i>	Thistle	July–Aug	NP	dmc
<i>Tagetes erecta</i>	Marigold	Jan–March	Np	dmc
<i>Helianthus sp.</i>	Wild Sunflower	Apr–June	NP	dmc
<i>Calendula officinalis</i>	Calendula	Feb–March	Np	dmc
Convolvulaceae				
<i>Ipomoea palmata</i>	Railway creeper	July–Aug	NP	dcm
Caricaceae				
<i>Carica papaya</i>	Papaya	July–Aug	Np	fdcm

Table 12.2 (continued)

Family Plant species	Common name	Flowering period	Source	Bee species
Cruciferae				
<i>Brassica campestris</i>	Sarson	Jan–March	NP	dfcm
<i>B. campertris</i>	Toria	Oct–Nov	NP	dfcm
<i>B. oleracea</i>	Cauli flower	Feb–March	Np	dfcm
<i>B. rapa</i>	Turnip	Feb–March	NP	dfcm
<i>Raphanus sativa</i>	Raddish	Feb–March	Np	dfcm
<i>Eruca sativa</i>	Taramira	Feb–March	NP	dfcm
<i>Iberis amara</i>	Candytuff	Jan–March	NP	dif
Cucurbitaceae				
<i>Cucumis melo</i>	Muskmelon	Apr–June	NP	dfcm
<i>Cucumis sativus</i>	Khira	May–Sept	NP	dfcm
<i>Citrullus fistulosus</i>	Tinda	Apr–Oct	NP	dfcm
<i>Citrullus lanatus</i>	Watermelon	Apr–June	NP	dfcm
<i>Cucurbita pepo</i>	Chapankadu	April–June	N	dfcm
<i>Luffa acutangula</i>	Kali tori	May–Oct	NP	dfcm
<i>Momordica charantia</i>	Karela	Apr–July	NP	dfcm
<i>Cucurbita moschata</i>	Pumpkin	Apr–July	NP	dfcm
Euphorbiaceae				
<i>Euphorbia pulcherrima</i>	Poinsettia	Dec–Jan	NP	dfcm
<i>Pyllanthus emblica</i>	Amla	Jul–Aug	NP	dc
<i>Ricinus communis</i>	Castor Plant	Aug–Sept	NP	dfcm
Graminae				
<i>Zea mays</i>	Maize	July–Aug	P	dmcf
<i>Sorghum vulgare</i>	Sorghum	July–Aug	P	dmcf
<i>Pennisetum typhoides</i>	Bajra	June–Aug	P	dmcf
Iridaceae				
<i>Gladiolus</i> sp.	Gladiola	May–July	NP	dm
Labiatae				
<i>Plectranthus rugosus</i>	Shain	Aug–Oct	NP	mc
Leguminosae				
<i>Acacia modesta</i>	Phalahi	Mar–April	P	dmcf
<i>Butea monosperma</i>	Palas	Mar–April	P	dc
<i>Canjanus cajan</i>	Arhar	April–May	NP	dcm
<i>Cicer arietinum</i>	Gram	Feb–March	NP	dcm
<i>Lathyrus odoratus</i>	Sweet pea	Jan–April	NP	dcm
<i>Medicago sativa</i>	Lucerene	Mar–May	NP	dcfm
<i>Parkinsonia aculeata</i>	Jerusalem thorn	Mar–May	NP	dmcf
<i>Pisum arvense</i>	Field pea	Feb–March	N	dmc
<i>Phaseolus mungo</i>	Moong	Aug–Sept	N	dmc
<i>Trifolium alexandrium</i>	Berseem	Mar–April	NP	dmcf
<i>Dalbergia sissoo</i>	Shisham	Mar–April	N	dmcf
<i>Trigonella foenumgraecum</i>	Methi	Mar–April		dmcf
<i>Alibizza Lebbek</i>	Siris	May–June	N	dmcf
<i>Tamarindus indica</i>	Imli	June–July	N	dmc
<i>Casia fistula</i>	Amalta	Apr–July	P	dmc
<i>Vigna unguiculata</i>	Cowpea	May–Sept	NP	dmc
<i>Crotalaria juncea</i>	Sanhemp	Sep–Oct	N	dmc
<i>Pisum sativum</i>	Mattar	Jan–March	NP	dmc
<i>Vigna mungo</i>	Mash	Aug–Oct	N	dmc
<i>Robinia pseudoacacia</i>	False kikar	May–June	N	dmc

Table 12.2 (continued)

Family Plant species	Common name	Flowering period	Source	Bee species
Liliaceae				
<i>Allium cepa</i>	Onion	May–June	NP	dmcf
<i>A. sativum</i>	Garlic	May–June	NP	dmcf
<i>Asparagus officinalis</i>	Asparagus	May–Nov	NP	dmcf
<i>Yucca savita</i>	Adam's needle	May–June	NP	d
Linaceae				
<i>Linum uistatissimum</i>	Linseed	Feb–March	N	dmcf
Loganiaceae				
<i>Buddleja asiatica</i>	Buddelia	Mar–April	NP	d
Lythraceae				
<i>Lawsonia intermis</i>	Mehandi	May–Sept	NP	dmf
<i>Lagerstromia indica</i>	Pride of India	Mar–July	P	dmcf
<i>L. speciosa</i>	Queen flower	May–Sept	P	dmcf
Magnoliaceae				
<i>Magnolia grandiflora</i>	Champa	Aug–Sept	P	dmcf
Malvaceae				
<i>Althea officinalis</i>	Holly hock	Apr–July	NP	dmcf
<i>Hibiscus esculentus</i>	Bhindi	Apr–Sept	NP	dmcf
<i>Malva sylvestris</i>	Sochal	Feb–March	NP	dm
<i>Gossypium hirsutum</i>	Narma	Aug–Oct	NP	dmcf
<i>G. arboretum</i>	Kapas	Aug–Oct	NP	dmcf
Meliaceae				
<i>Cedrella ciliata</i>	Tun	May–June	N	dmc
<i>Azadirachata indica</i>	Neem	Apr–May	N	
<i>Cedrella toona</i>	Tun	May–June	N	dmc
<i>Melia azadarch</i>	Drek	Mar–Arpil	N	
Mimosae				
<i>Acacia indica</i>	Kikar	July–Aug	P	dmcf
<i>A. catechu</i>	Khair	May–July	P	dmcf
Moraceae				
<i>Morus alba</i>	Toot	Feb–March	P	dmcf
Moringaceae				
<i>Morgina oleifera</i>	Sohanjana	Mar–April	NP	dmc
Musaceae				
<i>Musa paradisiaca</i>	Banana	Sept.Oct	NP	dmc
Myrtaceae				
<i>Psidium guajava</i>	Guava	May–June	NP	dmcf
<i>Syzygium cumini</i>	Jamuna	May–June	NP	dmcf
<i>Eucalyptus</i> sp.	Safeda	Mar–May	NP	dmcf
<i>Callistemon lanceolantus</i>	Bottle brush	Mar–April	N	dmcf
Nyctaginaceae				
<i>Boerhavia repens</i>	Itasit	July–Aug	P	dmcf
<i>Bougainvillea</i> sp.	Bougainvillea	Apr–May	P	df
Nymphaeaceae				
<i>Nymphea</i> sp.	Lilopher	May–July	P	dmc
Papaveraceae				
<i>Papaver shoeas</i>	Field poppy	Feb–March	P	dmcf
<i>Papaver somniferum</i>	Opium	Feb–March	P	dmc
Pedaliaceae				
<i>Sesamum indicum</i>	Til	July–Aug	N	dmc

Table 12.2 (continued)

Family Plant species	Common name	Flowering period	Source	Bee species
Polemoniaceae				
<i>Phlox drummondii</i>	Palox	Jan–Feb	N	dmcf
Portulacaceae				
<i>Portulaca grandiflora</i>	Portulaca	July–Aug	P	dmcf
Ranunculaceae				
<i>Delphinium denudatum</i>	Larkspur	Mar–April	P	dmcf
<i>Ranunculus bulbosum</i>	Buttercup	Feb–March	P	dm
Rahamnaceae				
<i>Ziziphus jujube</i>	Ber	June–Sept	NP	dmcf
<i>Z. mauritiana</i>	Ber	June–Nov	NP	dmcf
<i>Z. nummularia</i>	Kokan Ber	June–Sept	NP	dmcf
Rosaceae				
<i>Eriobotrya japonica</i>	Loquat	Jan–Feb	NP	dmcf
<i>Fragaria</i> sp.	Stwaberry	Mar–April	NO	dmcf
<i>Prunus persica</i>	Aru	Jan–Feb	NP	dfcm
<i>P. communis</i>	Pear	Feb–Match	NO	dfcm
<i>P. Saliciana</i>	Aloucha	Mar–April	NP	dfcm
<i>Pyrus malus</i>	Apple	Jan–Feb	NP	dfcm
<i>P. poshia</i>	Kanth	Mar–April	NP	dfcm
<i>Rosa mochata</i>	Gulbari	Mar–April	NP	dfcm
<i>Rubus ellipticus</i>	Akhray	Mar–April	NP	dfcm
<i>Rosa</i> sp.	Roses	Jan–Dec	Np	dfcm
Rubiaceae				
<i>Mitragyana paryfolia</i>	Kadam	Jan–Aug	P	dmfi
<i>Hamelia patentes</i>	Hamelia	July–Nov	P	dfcm
Rutaceae				
<i>Citrus limetoides</i>	Sweet lime	April May	NP	dfcm
<i>C. aurantium</i>	Sour orange	April–May	Np	dfcm
<i>C. sinensis</i>	Orange	April–May	NP	dfcm
<i>C. paradisi</i>	Grape fruit	Mar–April	Np	dfcm
<i>C. reticulata</i>	Kinnow	Mar–April	NP	dfcm
<i>C. medica</i>	Khatta	Mar April	NP	dfcm
Salicaceae				
<i>Salix acutifolia</i>	Bains	Feb–March	P	dfcm
Sapindaceae				
<i>Litchi chinensis</i>	Litchi	April May	NP	dfcm
<i>Sapindus detergens</i>	Reetha	April–May	NP	dfcm
Scophulariaceae				
<i>Antirrhinum majus</i>	Dog Flower	Mar–April	NP	dmc
Solanaceae				
<i>Capsicum frutescens</i>	Mirch	July–Aug	P	dmc
<i>Datura spemonium</i>	Datura	Apr–Sept	P	demc
<i>Lycopersion Esculentum</i>	Tomato	May–Sept	P	dfcm
<i>Solanum melongena</i>	Brinjal	Mar–Sept	P	dmc
<i>Nicotiana Pulmbagnifera</i>	Jangh tobacco	Feb–March	P	dmc
Tiliaceae				
<i>Grewia</i> sp.	Dhaman	June–July	P	dm
Umbelliferae				
<i>Coriandrum savitum</i>	Coriander	April–May	NP	dfcm
<i>Foenclum vulgare</i>	Fennel	April–May	Np	dfcm
<i>Daucas carota</i>	Carrot	April–May	NP	dfcm

Table 12.2 (continued)

Family Plant species	Common name	Flowering period	Source	Bee species
<i>Apium graveolens</i>	Celery	Mar–April	P	dfcm
<i>Ammi majus</i>	Honey plant	Mar–April	NP	dfcm
Urticaceae				
<i>Canabis sativa</i>	Bhang	July–Aug	P	d
Vailaceae				
<i>Viola odorata</i>	Bunafsha	Mar–April	N	dfcm
Vitaceae				
<i>Vitis vinifera</i>	Grape	Mar–April	NP	dfcm
Verbenaceae				
<i>Vinex negundo</i>	Banah	May–Aug	Np	dfcm
<i>Lantana camra</i>	Lantana	Mar–Sept	NP	dfcm
Zygophyllaceae				
<i>Tribulue terrisrtis</i>	Puncutre vine	May–June	NP	dfcm

d dorsata, *f* florea, *c* cerana, *m* mellifera

12.4.1 Assessment of Areas for Beekeeping

Productive beekeeping depends on good colony management and good beekeeping areas, and in order to promote it as a profitable agricultural occupation, areas with a good potential for beekeeping must be located and evaluated. One major problem in this respect is how to select sites for assessment. The following guidelines for the exploration and evaluation of potential beekeeping areas may be found useful:

1. Refer to lists of known major honey plants in other countries or regions with similar vegetation patterns, agro-ecosystems, climate and edaphic conditions; determine whether similar plants are to be found in the area under study.
2. The seasonal occurrence, in unusually high numbers, of wild (feral nests) of native honeybees can often indicate that there is ample forage in the area, at least during the period in question.
3. The mere presence of flowering trees and shrubs in limited numbers, or of a few hectares of land covered with good honey plants preferred by bees, does not necessarily indicate that the area has potential for commercial beekeeping.
4. Large scale beekeeping operations require large areas, usually hundreds or thousands of hectares of nearby land bearing good forage with high population densities. Good honey plants are characterized by relatively long blossoming periods, generally in terms of several weeks or months; high density of nectar-secreting flowers per plant or unit area; good nectar quality with high sugar concentrations; and good accessibility of the nectaries to the bees. The foraging land should be well proportioned, in terms of length and width, so as to promote foraging efficiency.
5. The supporting capacity of an area for honey production is best determined by monitoring weight changes in the bee colonies. Among other factors that affect the economic value of an area for beekeeping are average hive yields, prevailing honey prices in the area, as well as costs of colony-management inputs.

6. The fact that a flower is brightly coloured or that it has a strong scent does not always indicate that it is good for bees, unless the fact is confirmed by the criteria set out above.
7. The large-scale planting of honeybee forages has never been proved to be a profitable approach in terms of net economic return, except in integration with other agricultural activities, such as reafforestation, roadside plantings, animal pasture, etc.

12.5 Foraging Behaviour of *Apis cerana*

The individual bees quickly changed from nectar collection to pollen collection and vice-versa, in accordance with their colony needs. The proportion of pollen foragers and the amount of pollen collected increased with the amount of brood present. When colonies were deprived of brood, they foraged less and many former pollen gatherers shifted to nectar collection. Irrespective of the presence or absence of brood, absence of queen increased the nectar collection and decreased pollen collection (Free 1967). Bisht and Pant (1968) reported that *A. cerana* gathered pollen pellets throughout the year under conditions in Delhi city. The highest pollen gathering activity was recorded during January and March, whereas May–June were the periods of lesser activity. Rangarajan et al. (1974) studied the foraging behaviour of *A. cerana* and *A. florea* on *Helianthus annuus* L. bloom and noticed maximum activity from 0600 h to 1000 h, whereas 1200 h–1430 h was the period of limited activity. Naim and Phadke (1976) divided the annual foraging cycle of *A. cerana* season-wise and found that January–March was peak period of pollen collection, while honey storing activity was at its peak during March and April. Naim and Bisht (1978) reported that January–March was peak period of pollen collection and honey-storing activities were at peak during March and April. Tanda and Goyal (1979a, b) observed the peak period of pollen collection by *A. cerana* and *A. mellifera* in the morning hours on *Gossypium* spp. They conclude that no bees from either species collected mixed pollen on all foraging trips on the same day. Pollen availability was maximum in the morning and decreased in the afternoon, consequently some pollen foragers of both the species shifted to nectar collection and they assumed pollen collection next day morning.

Maximum foraging activity of the Indian honey bee, *A. cerana*, was noticed in the month of July and minimum activity during January. More number of pollen plus nectar collectors was recorded than pollen or nectar collectors alone. It was also observed that there was greater variation among pollen collectors over nectar or water collectors (Reddy 1980). Singh (1989) found that pollen was collected throughout the year by *A. cerana* at Saharanpur (U. P.) with maximum activity during October. The second peak was recorded during February and April. From June to October pollen foragers started their activity at 0600 or 0700 h, but in November and December pollen foraging did not commence till 1000 h. The foraging activity began at 1400 h and 1800 h in December and July respectively.

Foraging activity was directly proportional to the increasing ambient temperature and also with increasing radiation up to certain level. However, it did not appear to be correlated with changes in atmospheric humidity and pressure (Burrill and Dietz 1981). Thakur et al. (1982) reported that at noon, the foraging activity was maximum (300 bees returning in 5 min) and there were more pollen foragers in the morning than during afternoon.

Foraging activity was greater during 0800–1200 h followed by 1700 h–1800 h. The number of nectar gatherers was high in the early morning (0600–0800 h) or from 1200–1600 h, whereas the number of pollen gatherers was highest in the late morning (0900–1200 h), decreased in the afternoon and there was a sharp increase between 1700–1800 h (Reddy 1983). Bhalla et al. (1983) reported that honey bees started foraging after 0900 h and were most active from 1100 h to 1600 h. Honey bees gathered pollen and nectar throughout the year irrespective of climatic conditions. The number of pollen foragers was highest between 0900 and 1200 h, while the number of nectar foragers was highest between 1000 h and 1500 h. There was little foraging before 0600 h and after 1800 h. Pollen foraging was highest during February and March as well as July and October (Verma 1983).

Gupta et al. (1984a) studied the foraging activity of *A. cerana* and *A. mellifera* on *plectranthus* flowers at Rampur (Himachal Pradesh) and noticed the variation in the rate of foraging activity during different day hours. Maximum number of pollen gatherers of *A. cerana* was seen during 0700–0900 h, while nectar collection activity reached the peak at 1200 h, whereas *A. mellifera* showed peak pollen collection activity between 0900 and 1000 h. The peak period of foraging activity of *A. cerana* was in the month of May and June. Foraging data showed the varying peak activity during different seasons. In summer, peak activity was at 0800 and 1000 to 1600 h, during rainy season at 0900 and 1000 h, in autumn between 0900 and 1000 to 1200 h, in early winter between 0900 and 1400 h, in late winter at 1100 h and in spring season between 0800 and 1100 h. The percentage of nectar collectors were greater than pollen or pollen plus nectar collectors in all the seasons of the year and greater seasonal variations were observed in the percentage of nectar collectors as compared to other categories of foraging bees (Mattu and Verma 1985). Verma and Chauhan (1985) observed the maximum foraging activity of *A. cerana* between 1100 and 1200 h and again between 1400 and 1500 h. Verma and Dulta (1986) compared the foraging behaviour of *A. mellifera* and *A. cerana* on apple flowers. *Apis cerana* workers started their activity significantly earlier in the morning than *A. mellifera* and their activity ceased late in the evening hours. Abrol and Bhat (1987) observed that foraging activity of *A. cerana* was positively and significantly correlated with temperature and non-significant with relative humidity. However, the cessation of foraging activity seemed to be independent of atmospheric temperature and relative humidity.

Dustmann and von der Ohe (1988) have reported the direct correlation of flight activity of *A. mellifera* with the maximum day temperature. The maximum foraging activity of *A. cerana* occurred during December and January and a second peak activity during February. Peak pollen collection tended to coincide with those of nectar collection (Anonymous 1989). Virakthamath (1990) studied the foraging profile of *A. cerana* in Raichur, Karnataka. He observed major pollen foraging (80 %) before

noon and foraging throughout the day with a major peak during 0600 and 1100 h and minor peak during 1600 to 1800 h. He further observed more number of pollen and nectar foragers during August and February and August and March respectively, with a dearth period during May and July. Sattagi and Lingappa (1993) reported that peak foraging activity of *A. cerana* was between 0800 h and 1300 h. during monsoon season (June–September), 0800 and 1100 h. in winter season (October–January), 0800 and 1100 h. and 1600–1800 h. during summer season (February–March). During remaining hours of the day, the activity was low in different season at Dharwad, Karnataka, India.

The visits of *A. cerana* to mango flowers gradually increased from 0600 to 1200 h. The peak activity was noticed between 0900 to 1100 h (16–20 bees per panicle per 5 min). The bee visits decreased from 1400 to 1700 h (2–5 bees per panicle per 5 min) and no visits were observed after 1800 h. The bee activity was low in the beginning, but picked up gradually to reach the peak at flowering from late February to early March (60–95 bees per panicle per 5 min) at Bangalore (Jyothi 1994). Singh and Singh (1997) reported that *A. cerana* was main foragers (46.37 %) followed closely by *A. dorsata* (41.99 %). The peak foraging activities was noticed between 0600–0900 h and that of later was between 0700–0900 h and 1500–1700 h on sunflower at Manipur, India.

Holi and Viraktamath (1997) reported that foraging behaviour was more or less similar in monsoon and winter with a peak activity of outgoing foragers, pollen and nectar foragers during 1100–1300 h. In summer, there were two distinct peaks as against only one during monsoon and winter season. A major outgoing and pollen foragers occurred between 0700–0800 h and a minor peak between 1700–1800 h. The nectar foragers were maximum between 0700–1000 h and 1700–1800 h. Foraging activities had positive correlation with the temperature and negative correlation with the rainfall and RH. Chowde Gowda et al. (2005) reported that in *A. cerana* number of pollen foragers was more during 9.00–11.00 h; nectar foragers were maximum during 13.00–16.00 h of the day. The number of nectar foragers was maximum during January and April and pollen foragers were maximum during November and December. Banakar (2009) reported 87 plants for *A. cerana* under Dharwad conditions, Karnataka, India as sources of nectar and pollen (Table 12.3).

12.5.1 Brood Rearing Activity

Ramachandran (1939) studied the brood rearing in *A. cerana* Fab. and reported egg laying by the queen varied between 300–500 eggs per day. Rahman and Sharma (1945) observed a high rate of brood area during the winter months at Lyallpur (Pakistan). Sharma (1948) again at Lyallpur studied variation in brood area during different seasons. Brood rearing was observed in winter season also and a slight decline in the latter half of March, after which brood rearing was carried out at almost the same rate. Practically, none of the colonies reared brood continuously throughout summer. He recorded up to 1,800 cm² of brood (24,816 cells) amounting to 1,240

Table 12.3 List of pollen and nectar sources of *A. cerana*. (Banakar 2009)

Serial no.	Botanical name and family	Common name in English	Local name	Flowering period	Pollen/nectar/both
1	<i>Acacia auriculiformis</i> (Fabaceae)	Earleaf acacia	Sabu gida	May–October	P
2	<i>Acacia catechu</i> (Fabaceae)	Cutch tree	Kachu	May–October	P
3	<i>Acacia concinna</i> (Fabaceae)	Soap pod	Sige	September–November	P and N
4	<i>Ailanthus triphysa</i> (Simarubaceae)	Stink tree	Maddi dhoopa	February–March	P and N
5	<i>Allophylus cobbe</i> (Sapindaceae)	Sidisale	Moorele balli	April–June	N
6	<i>Alseodaphne semicarpifolia</i> (Lauraceae)	Karuvadi	Mashe	March–July	N
7	<i>Alstonia scholaris</i> (Apocynaceae)	Dita bark tree	Maddale	March–April	N
8	<i>Anacardium occidentale</i> (Anacardiaceae)	Cashew nut	Geru mara	December–January	N
9	<i>Anthocephalus cadamba</i> (Rubiaceae)	Kadamba tree	Kadamba vruksha/apathina mara	April–June	N
10	<i>Aporosa lindleyana</i> (oleaceae)	Indian olive	Salle mara	January–March	P
11	<i>Archidendron monadelphum</i> (Fabaceae)	Kachlora Kan	Karinje/Nuggikar	January–February	N
12	<i>Areca catechu</i> (Arecaceae)	Betel nut	Adike	May–December	N
13	<i>Artocarpus heterophyllus</i> (Moraceae)	Jack fruit	Halasina mara	January–March	P
14	<i>Artocarpus hirsute</i> (Moraceae) Wild	Jack fruit	Hebbalasu	December–March	P
15	<i>Artocarpus lakoocha</i> (Moraceae)	Lakooch	Naate huli mara	January–February	P
16	<i>Bombax ceiba</i> (Bombaceae)	Yellow Silk	Cotton Tree Boorala	January–March	P and N
17	<i>Butea monosperma</i> (Fabaceae)	Flame of the forest	Muttaga	February–March	N
18	<i>Calophyllum apetalum</i> (Clusiaceae)	coachwood Babbi		October–November	P
19	<i>Canarium strictum</i> (burseraceae)	Black dammer tree	Kayi dhoopa	January–March	P and N
20	<i>Canthium dicoccum</i> (Rubiaceae)	Kumar chikni	Hanigere	April–August	N
21	<i>Carallia brachiata</i> (Rhizophoraceae)	Indian oak	Andi/anda murugals	December–February	N
23	<i>Careya arborea</i> (Melastomataceae)	Ceylon oak	Kavalu	February–April	P and N
24	<i>Caryota urens</i> (Arecaceae)	Indian sago palm	Baine	January–December	P
25	<i>Cassine glauca</i> (Celastraceae)	Marble tree	Kannur mara/Gotadike mara	February–June	P

Table 12.3 (continued)

Serial no.	Botanical name and family	Common name in English	Local name	Flowering period	Pollen/nectar/both
26	<i>Celtis philippensis</i> (Ulmaceae)	Sugar hackberry	Peenari/Hetari mara	January	N
27	<i>Chionanthus malabarica</i> (Oleaceae)	Malabar Fringe Tree	Akkerakalu	January–February	N
28	<i>Chrysobalanus roxburghii</i> (Sapotaceae)	The star Apple	Hale mara	April–May	P and N
29	<i>Cinnamomum malabathrum</i> (Lauraceae)	Lavangada yele	Neeshne	January–June	N
30	<i>Cinnamomum zeylanicum</i> (Lauraceae)	Wild cinnamontree	Dalchinni	December–March	N
31	<i>Cocos nucifera</i> (Pandanaeae)	Coconut	Tengina mara	January–December	P
32	<i>Cordia dichotoma</i> (Boraginaceae)	Sebsten plum	Challe kayi	March–April	P
33	<i>Dalbergia latifolia</i> (Faboidaeae)	Indian rose	Wood Beete/Sisam	January–March	N
34	<i>Dillenia pentagyna</i> (Dilleniaceae)	Karmal Gudde	Kanagila	January–March	P and N
35	<i>Diospyros malabarica</i> (Ebanaceae)	Gaub tree	Antina mara	March–May	N
36	<i>Diospyros melanoxyton</i> (Ebanaceae)	Coromandele-bony	Tumari	April–May	P
37	<i>Diospyros microphylla</i> (Ebanaceae)	Gaub Sannale	Mara	January–March	N
38	<i>Diospyros montana</i> (Ebanaceae)	Mountain ebony	Bala gane	April–May	N
39	<i>Emblia officinalis</i> (Euphorbiaceae)	The emblic Myrabolan	Nelli	February–March	P and N
40	<i>Eucalyptus citriodora</i> (Myrtaceae)	Eucalyptus	Niligeri mara	October to February	P and N
41	<i>Flacourtia montana</i> (Flacourtiaceae)	Mountain Sweet	Thorn Sampige hannu	November–January	P
42	<i>Garcinia gummigutta</i> (Clusiaceae)	Malabar gamboges		Uppage February–March	N
43	<i>Garcinia indica</i> (Clusiaceae)	Indian gamboges	Murugalu	April–August	N
44	<i>Grewia tiliaefolia</i> (Tiliaceae)	Dhaman	Dadasalu mara	February–April	N
45	<i>Holigarna arnottiana</i> (Anacardiaceae)	Jungle marking nut	Holageru	January–February	P and N
46	<i>Holigarna beddomei</i> (Anacardiaceae)	Beddomes	Black varnish Doddale holegeru	January–February	P and N
47	<i>Holigarna ferruginea</i> (Anacardiaceae)	Holegeru		March–May	P and N
48	<i>Hopea ponga</i> (Dipterocarpaceae)	Haiga		March–April	N
49	<i>Ixora brachiata</i> (Rubiaceae)	Gorbale	Gorbale	November–January	P and N

Table 12.3 (continued)

Serial no.	Botanical name and family	Common name in English	Local name	Flowering period	Pollen/nectar/both
50	<i>Lagerstroemia lanceolata</i> (Lythaceae)	Ben tree	Nandi mara	March–May	P and N
51	<i>Mammea suriga</i> (Clusiaceae)	Surangi	Suragi mara	March	P and N
52	<i>Mangifera indica</i> (Anacardiaceae)	Mango tree	Mavina mara	January–March	N
53 N	<i>Mitragyna parvifolia</i> (Rubiaceae)	Kaim	Etagalu mara	May to July	P and
54	<i>Moringa oleifera</i> (Moringaceae)	Drum stick	Nugge gida	February	P
55	<i>Murraya koenigii</i> (Rutaceae)	Curry leaf to tree	Curry leaf plant	March–May	N
56	<i>Musa paradisiacal</i> (Musaceae)	Banana	Bale	January–December	P and N
57	<i>Nothopogia racemosa</i> (Anacardiaceae)		Gandu holegeru	April–May	P and N
58	<i>Peltoforum ferrugineum</i> (Fabaceae)	Copper pod	Tamra gida	March–July	P
59	<i>Phoenix sylvestris</i> (Arecaceae)	Wild date palm	Echalu mara	February–May	P
60	<i>Phyllanthus emblica</i> (Euphorbiaceae)	The emblic myrabolan	Nelli	February–March	P and N
61	<i>Pongamia pinnata</i> (Faboideae)	Indian beech tree	Honge mara	MarchMay	N
62	<i>Randia dumetorum</i> (Rubiaceae)	Bush randia	Khare mara	April–June	P
63	<i>Samanea saman</i> (Fabaceae)	Rain tree	Male mara	April–May	P
64	<i>Santalum album</i> (Euphorbiaceae)	Sandal wood tree	Chandana/Shri gandha	May–June	N
65	<i>Sapindus emarginatus</i> (Sapindaceae)	Soap nut	Antuvala	November–February	P and N
66	<i>Schleichera oleosa</i> (Sapindaceae)	Ceylon oak	Sagadi mara	February–March	N
67	<i>Strychnos nuxtomica</i> (Loganiaceae)	Snake wood	Kasarka	February–April	P and N
68	<i>Syzygium caryophyllaeum</i> (Myrtaceae)		Kunta neralu	February–June	P and N
69	<i>Syzygium cumini</i> (Myrtaceae)	Indian cherry	Nerale	February–May	P and N
70	<i>Syzygium gardneri</i> (Myrtaceae)	Henneralu		March–April	P and N
71	<i>Terminalia bellerica</i> (Combretaceae)	Behera	Tari mara	February–April	N
72	<i>Terminalia chebula</i> (Combretaceae)	Gall nut	Andle kayi	March–April	N
73	<i>Terminalia paniculata</i> (Combretaceae)	Flowering murdah	Honagalu	July–December	P and N
74	<i>Terminalia tomentosa</i> (Combretaceae)	Laurel	Matti mara	May	P and N
75	<i>Xylia xylocarpa</i> (Rosaceae)	Burma iron wood	Jambe mara	March–April	P

Table 12.3 (continued)

Serial no.	Botanical name and family	Common name in English	Local name	Flowering period	Pollen/nectar/both
76	<i>Zanthoxylum rhetsa</i> (Simarubaceae)	Indian prickly ash	Jammana kayi	June–November	N
77	<i>Ziziphus rugosa</i> (Phamnceae)	Wild jujuba	Bile mullu hannu	January–April	N
Herbs and shrubs					
1	<i>Anisomeles indica</i> (Lamiaceae)	Indian catmint	Karithumbi	November–January	P and N
2	<i>Antigonon leptopus</i> (Polygonaceae)	Coral creeper	Antigonum	January–December	N
3	<i>Chromolaena odorata</i> (Euphorbiaceae)	Communist weed	Durge soppu	November–February	N
4	<i>Cleome chelidonii</i> (Capparaceae)	Celandine spider flower	Habbu balli	June–October	P and N
5	<i>Hyptis suaveolens</i> (Lamiaceae)	American mint		September–November	P and N
6	<i>Mimosa pudica</i> (Mimosaceae)	Touch me not	Muttidare muni	January–December	P and N
7	<i>Ocimum americanum</i> (Lamiaceae)	Hoary basil	Jangli tulsi	August–October	P and N
8	<i>Ocimum basilicum</i> (Lamiaceae)	Basil Tulsi		August–October	P and N
9	<i>Tribulus terrestris</i> (Zygophyllaceae)	Puncture wine	Negil-mullu	January–SDecember	P and N
10	<i>Tridax procumbens</i> (Asteraceae)	Tridax daisy	Shaavanthi	January–December	P and N

P Pollen source, *N* Nectar source, *P, N* Both Pollen and Nectar source

eggs per day during February. Ramachandran and Mahadevan (1950) observed a steady decline in brood rearing from June to September at Coimbatore (South India). There was an initial expansion of brood in November and December, and the peak brood rearing was noticed during January and March.

Ramachandran and Cherian (1952) studied the brood rearing cycles of *A. cerana* at Coimbatore, India. They reported 692 cm² or 10,799 cells of brood in a thriving colony at the peak brood-rearing period. This gave an average output of 514 eggs per day. Kapil (1957) Studies of the brood rearing activity of *A. cerana* at Allahabad, Uttar Pradesh, revealed 6,178 and 878 sealed cells for a period of 12 days during February and April respectively, suggesting an average oviposition rate of 515 and 681 eggs per day, respectively. Sharma (1958b) compared the brood-rearing activity of the mountain grey race of *A. cerana* in the plains and at higher altitudes. He concluded that the brood rearing activity was suspended at higher altitudes with the approach of winter, but it did not happen in the plains.

The maximum egg laying rate of the mountain grey race of queen bees was about 1000 eggs per day, whereas the golden race of the plains laid about 600 eggs per day.

Bisht (1966) found that maximum brood rearing activity of *A. cerana* in February and March at Delhi and December was quite unfavourable. Saraf and Wali (1972) calculated the egg laying rate of *A. cerana* (hill strain) in Kashmir by measuring the brood area at every 3 weeks. Brood rearing was maximum in May and early June and declined in October. According to Reddy (1980) who made observation on the annual cycle of brood rearing by *A. cerana* at Bangalore, brood rearing occurred throughout the year and this activity was maximum from March to November and minimum in December and January. The greatest amount of sealed brood was present in April. Shah and Shah (1981) studied the egg-laying capacity of *A. cerana* in Kashmir and concluded that the egg laying capacity of Kashmiri bees was higher in the *A. cerana* group in India and also higher than exotic *A. mellifera* L. Maximum brood rearing was reported in May and it was minimum in January. Bisht et al. (1982) reported from Delhi that brood rearing of *A. cerana* was highest in February–May with a minimum in December. Pollen storage was highest in April–July. Fukuda (1983) observed that during colony development early in the season, most food was brought during period of maximum population. The amount of pollen collected was related to the amount of brood. In this colony, total oviposition was 1.5×10^5 eggs.

In *A. mellifera*, Harbor (1986) observed that colonies of 4,500 bees population produced the most brood per bees population increased above 4,500 bees, brood production per bee decreased. However, during summer dearth, the colonies of 9,000 bees population produced the most brood per bee. Overall optimal colony size was 9,000 bees under Louisiana, USA condition. Dustman et al. (1988) reported that cold snaps prevent flight activity which resulted in an interruption of pollen intake, low foraging activity also had a negative effect on brood rearing by increasing brood cannibalism. Accordingly, the nurse bees population in next generation was reduced. Due to an insufficient supply of pollen during larval and early imaginal stage of the nurse bees, hypopharyngeal glands were not adequately supplying open brood with worker jelly. Rana and Goyal (1994) reported that in May there was a maximum brood area of $3,225 \text{ cm}^2 + 21.6$ and the minimum brood area during January is $1,371 \text{ cm}^2$ brood area in *A. cerana*. In winter, Indian bee construct more brood area as compared to exotic species. Verma (1998) reported that the average brood area was maximum in October followed by March, April, November and September; this shows that the brood area was directly related to the availability of pollen grains.

12.5.2 Pollen Stores in the Brood Chamber

Ramachandran and Mahadevan (1950) reported that *A. cerana indica* stored greater amount of honey and pollen in February and April months at Coimbatore in Tamil Nadu, India. Naim and Phadke (1976) reported that maximum honey storing activity of *A. cerana* was during March and April at Pusa (Bihar). Reddy (1980) reported greater amount of honey and pollen stores in February and April at Bangalore (Karnataka). Hellmich and Rothenbuler (1980) reported that the difference of pollen hoarding was not found between the line of high pollen hoarding behaviour (HPH)

and the line of low pollen hoarding behaviour (LPH) when brood was in the egg stages and pollen stores were small, but more abundant difference in the lines during larval stage were maintained after brood cells were capped. However, the amount of pollen stored did not change significantly. HPH bees also hoarded more pollen in the absence of brood. The two lines used similar amount of pollen and reared similar amounts of brood. Mortality was higher in LPH bees than that of HPH bees. Bees which hoard a large amount of pollen are either less inhibited from collecting pollen by the presence of stored pollen or more stimulated to collect pollen by its absence.

Bisht et al. (1982) reported that *A. cerana* stored highest number of cells with pollen in the month of April, May, June and July in the order mentioned least in the month of December. Pollen storage was positively correlated with the dimension of the bee hive. Many pollen stored honey cells were observed during April and December. Whereas, in January, February and March the stored honey cells was less than the expected numbers. Mattu and Verma (1985) observed that honey and pollen stores were greater in summer and autumn than in other seasons in Shimla. Handal (1985) reported that the annual intake of pollen was 14.07 kg per bee colony in La liberated, EL Salvador. In the rainy season (May–October) the mean intake was 1,452 g, while in the dry season (November–April) it was 867 g. From August to April, the average rate of pollen consumption per 100 larvae was 3.01 g for every 21 days. Rahman and Rahman (1993) reported that in colonies of *A. cerana* the maximum pollen area was found to be $1,042.36 + 250.00 \text{ cm}^2$ in February and minimum pollen area was recorded to be $425.20 + 143.89 \text{ cm}^2$ in October. Rana and Goyal (1994) reported that a maximum pollen area of $648 + 9.5 \text{ cm}^2$ during December and minimum pollen area of $1.8 + 0.1 \text{ cm}^2$ during May in *A. cerana*.

12.5.3 Honey Yield

Ahmed et al. (1983) reported that the colonies of *A. cerana* from Swat and Margalla in Pakistan yielded on an average annual honey yield of 8.17 kg for the swat strain and 7.75 kg for the margalla strain. Liu Zui-Sheng (1984) reported that *A. cerana* collected 30 % less nectar during a foraging trip than *A. mellifera* and attributed this to its smaller size of body and honey sacs. The difference in the body size was speculated to ultimately affect the honey production potentials. According into Wongsiri et al. (1986), the native *A. cerana* colonies, produced 70 % less honey per colony annually than the exotic *A. mellifera* in the Guangdong province of china, where beekeeping with this native bee species is very popular and it represents 90 % of all bees. They also stated that since the colony size of *A. cerana* is 30 % smaller than the exotic bee *A. mellifera*, the individual worker bee of *A. cerana* is in no way inferior to *A. mellifera* in respect of honey production. Oldroyd and Goodman (1988) found that the hybrid queens did not lead to increased honey production. Szado and Lefkovitch (1989) noticed, during the honey flow period, hives which contain queens of 2 or 3 years old produced 120.2 (60.9–210.8) kg honey per colony. Pidek (1989) reported

that when queen was caged for a period of 24 days in early July it led to increase in honey and wax yield.

12.5.4 Pollen and Nectar Sources

Sharma and Nair (1965) made pollen analytical studies on 13 honey samples collected from Uttar Pradesh. The dominant honey plants represented by pollen in the honeys were the *Rumex* sp., *Nephelinum* sp., and other plants of the family Myrtaceae, Liliaceae, Rosaceae, Meliaceae, Brassicaceae and Euphorbiaceae. The pollen of *Antigonon leptopus* Hook and Arn. and *Moringa pterygosperma* Gaertn. were predominant in the honey of Lucknow (Nair and Singh 1965). Chaturvedi (1973, 1977) reported the high floral fidelity of *A. c. indica* by analyzing the pollen loads from Banthra. Chaudhary (1978) analysed 5,200 pollen loads from Indian honey bee, *A. cerana indica* and observed only 56 % of pollen from more than one plant species. The analysis of twelve different apiary honeys from eight different states (Maharashtra, Bihar, Jammu and Kashmir, Uttar Pradesh, Andhra Pradesh, Karnataka, Kerala and Tamil Nadu) indicated the presence of *Syzygium cumini* Skeels, *Terminalia chebula* Retz, *Nephelium litchi* Sonner, *Isodon regosus* Wall, *Brassica rugosus* Prain, *Phylla nodiflora* (L.) Greene, *Borassus flabellifera* L. *Schefflera* sp., *Sapindus emarginatus* Vahl, *Havea brasiliensis* (Muell) Arg., *Eucalyptus* spp and *Tamarindus indica* L. as dominant pollens in honeys (Seethalakshmi 1980).

Lieux (1980) analysed 54 honey samples in Louisiana and observed 23 species of plants as minor sources contributing 1.0–1.5 % pollen. Padagoan honey indicated the presence of *Schefflera venulosa* Harns. (Araliaceae), *Syzygium cumini* as dominant sporomorphs (Chaubal and Kotmire 1980). Dhaliwal and Atwal (1980) found that *A. florea*, *A. dorsata* and *A. mellifera* foraging on alfalfa (*Medicago sativa* L.) carried 60.0, 60.0 and 66.6 % pure pollen respectively. The melissopalynological analysis of apiary honey from Pauri Garhwal (U.P.) indicated the presence of 30 types of pollen. Among these, the major ones were Myrtaceae, Lamiaceae, Apiaceae and Poaceae groups (Gour and Nanwani 1989). In more than 400 honey samples from different regions of Brazil, the pollen spectra indicated that 190 of them were from monofloral honeys. The most common plant species for these honeys were *Eucalyptus* and *Citrus* species from crops and wild species that produce honey appreciated by humans were from Asteraceae and Mimosidae with different pollen types (Barth 1990). The analysis of pollen loads and honey collected from *A. cerana* colonies at west Godavari districts (Andhra Pradesh) has shown that nearly 50 % of pollen was from *Cocos nucifera* L. (Suryanarayan et al. 1990). Melissopalynological studies conducted on 21 honey samples collected from 10 localities of the North-East Himalayan region revealed the presence of dominant sporomorphs as *Brassica* sp., *Adhatoda* sp., *Clematis* sp., *Mussaenda* sp., *Helianthus* sp. and Papilionaceous, Rubiaceae, Rutaceae and Zingiberaceae members. Pollen analytical studies also indicated both unifloral and multifloral types of honey samples (Singh et al. 1994).

Kalpana and Ramanujam (1997) analysed 250 samples of honeys from different apiculture enterprises of three districts of Andhra Pradesh (Godavari, Krishna and Guntur) and reported that *Sapindus*, *Eucalyptus*, *Borassus*, *Anacardium* and *Cocos nucifera* L. (in east Godavari), *Borassus* and *Hygrophila* (in Krishna), *Phoenix*, *Sapindus*, *Borassus*, *Hygrophila*, *Mimosa* and *Cleome* (in Guntur) as major pollens in honeys.

Sattagi (1997) studied the bee–flora in and around Dharwad (Karnataka) and recorded 165 plants species as bee forage plants. Out of these, 12 were identified as major sources and also revealed that the major share was from field crops which have limited flowering period and do not supply food throughout the year which lead to the uncertainty of beekeeping in this area. Holli and Virakthamath (1997) reported that in total 132 plants species were found to yield pollen and nectar source, they constituted 26 field crops, 27 forest trees, 22 vegetable crops, 21 ornamental plants, 19 fruits and plantation crops and 17 herbs, shrubs and bushes in Dharwad region. Joshi et al. (1998) reported that eight of the honey samples were unifloral *Eucalyptus* spp and *Syzygium cumini* and predominant source, *Peltophorum pterocarpum* is a major source of nectar for *A. cerana*, *A. dorsata* and *Trigona iridipennis*. Vasudeva (2009) list out the flora of Sirsi taluka by conducting the survey and listed about 255 tree species, 131 shrubs and 40 herbs.

Information on different aspects of bee forage is essential for the efficient management of honeybee colonies. Management scheme for each apicultural region is closely correlated with the flowering of local honey and pollen producing plants as also the climatic conditions. Basic research in the area of forage ecology has been done and floral calendars for different regions have been prepared. On the basis of surveys, potential beekeeping areas have been identified.

The most serious problem for Indian beekeeping has been the decline in flora due to deforestation and clearing of wastelands for extensive agriculture. Improvement of bee flora is not possible by individuals efforts and a beekeeper has to adopt and adjust only to the cropping patterns of the area and forest wild flora available in the locality. Recently central and state Governments and local organisations have helped in expansion of planted areas of bee forage along highways, wastelands, etc. To get good results, plantation of selective trees and shrubs is essential and this should be done on the basis of multiple use principle including bee forage as one of the uses. Flowers of many plant species are visited by bees for nectar and/or pollen but relative importance depends on the quality and quantity of rewards available and also on the density of the plant species. Intensive research in this area has generated this type of information on many of the important flora. The knowledge accumulated can be made use of while planning plantations on the basis of accessibility of the potential bee forage areas and migration schedules can be worked out. Migratory beekeeping is practised by many commercial beekeepers in states like Himachal Pradesh, Bihar and south India but micro-regional survey of bee forage would be required for planning short and long distance migration schedules.

Honeybees have closed links with the flora because adults and young ones live solely on nectar and pollens. Usefulness of a flora depends upon the quantity of food and energy harvested and the energy requirement of bees. Nectar is secreted by nectaries and is usually a reward for the visitors bringing about pollination. In general,

the quantity of nectar secreted is directly related to the pollination requirements of the crop. Honey is made by bees from the nectar collected from floral and extrafloral nectaries. Sometimes honeydew is also an important source in certain localities, but the well-being of bees is greatly dependant on the value of the flora. For the selection of the apiary site, it is essential to know the plants, which provide nectar or pollen to bees. Data on different aspects can be gathered for couple of years to establish the potentials of an area for beekeeping. Scale colony provides useful information. Weight of the scale colony is regularly recorded and the changes in weight are correlated with the flowering plants which are being visited by bees and also the weather conditions. Flowers present nectar and pollen during specific time of the day, therefore, bee activity on the flora should be carefully recorded. Recording the bee activity at different hours during a day should give useful information. Melissopalynological studies are essential to ensure as to which plants are availed by bees. Pollen analysis of honey samples taken at different time of the year and comparing with reference slides give the exact information about the floral sources for bees in vicinity.

Bee flora should be studied from different angles to find out the value of bee forage though the total nectar production per flower is important. Some flowers secrete nectar only for one day and few others for short time and still there are flowers like *Schefflera wallichiana*, which continue nectar secretion for about a fortnight. The time for which nectar plant blossoms are another important point determining the value of flora. In some forages the duration between the start and end of flowering is very short, whereas in others like *Brassica* spp. there is a succession of flowering and it lasts for about a month. Trees present larger number of flowers as compared to bushes, shrubs and crop plants unless the latter are growing in large continuous areas. Concentration of nectar sugar gives an objective measure. Total sugars per flower per day is the sugar value which is normally estimated for such studies. Sugar value is the number of mg of sugar secreted by one flower over a period of 24 h. However, total quantity of nectar produced and the amount available to or harvested by bees give valuable information.

12.6 Nectar–Sugar Concentration

Nectar concentration in most bee forages varies between 20–50 % but may be as low as 6 % in *Bombax ceiba* L. to 15 % in pear and as high as 79 % in silver oak (*Grevillea robusta*). Nectar and sugar concentration (%) has been worked out for some flora in India. For example, *Grevillea robusta* had 79 % sugars in the nectar 23 plants including *Tecoma grandiflora* had 14 %, peach and pear 70 %, *Brassica juncea* 52 %, *Barberis* 48 %, some citrus spp. 40–44 %, *Sapindus detergens* 40 %, *Plectranthus* 38 %, *Cidrella cerata* 36 % *Carvia callosa* 35 %, *Thelepaepale ixiocephala* 35–64 %, *Impatiens balsamina* 16–25 %, *Nephelium litchi* 61–78 %, *Plectranthus* 26–54 % *Woodfordia floribunda* 10–12 %, *Brassica campestris* var. *toria* 38.5–53.5 % and onion 59–75.5 %. Gupta found that there were considerable differences in the amount of nectar sugar production in the flowers of different cultivars of cauliflower and in

the attraction of bees to them. Average nectar sugar contents varied from 0.035 to 0.150 mg/flower/24 h. On the basis of such studies, honey potentials per unit area are estimated for cultivated crop/fruit plants. For wild flora, the density per unit area is estimated by sampling and honey potentials of the flora in the area are worked out. Honey potentials of forage are only estimates but it serves as a useful guide. Honey potentials of forage are liable to change. This is happening fast in developing agriculture in India where changes in land-use patterns have been very frequent. Under extensive agriculture, vast wastelands are cleared for cultivation and this reduced the wild flora. Land may be put to urbanization or industrialization causing reduction in cultivated and wild bee flora. There are introductions and extension of cultivation of new crops like sunflower, safflower and other oil-seed crops and this has substantially changed the scenario with regard to beekeeping potentials. There are many agricultural practices, which affect the bee flora. Mechanized agriculture reduces the weed plants, which may be serving as bee forage. The use of weedicides is another agricultural practice, which reduced the weed forage for bees.

The nectar yielding plants contributing to nectar/honey flow are specific to different areas and they have definite micro-regional habitats. Even in rich floral areas, continuous succession of nectar yielding plants throughout the year is lacking. In some localities, there is single surplus honey flow and in good areas two surplus flows may be available. In north-western hills of Himachal Pradesh and Jammu and Kashmir, the lower and mid hills present spring, early summer flow and in areas like Kashmir and parts of Himachal Pradesh, there is rich flow from *Plectranthus* in autumn. Bees face protracted dearth period in winters and only subsistence flora is available in rainy season, but heavy downpours are hazardous to bees. In north Indian and Gangetic plains, major flora of Brassica is available from September through early February. This build up and surplus flow is followed by spring and summer surplus honey flow from *Eucalyptus*, *Dalbergia sissoo* and other trees and berseem is available by bees till May. Hard summers are also no flora availability period but some weeds and crops present subsistence forage in rainy season. In Western Ghats and South India, there is medium to major flora available from October to May and important sources being jamun, hirda, carvi, soapnut, rubber plant, *Schefflera*, etc. but June–September is a dearth period. Therefore, for beekeeping sub-tropics may have an inactive period of 1 or 2 months. In tropics it is dry season or excessive rainfall and in hills it is winter, which is troublesome to bees. Surplus flow season may vary from 1 or 2 and rarely 3 in a year.

Beekeeper, to maximize his honey crop, should have a thorough knowledge of the floral cycle, onset of major honey flow and dearth period. The bee colonies should be managed in a way so as to have maximum foraging strength to avail major flow and economical or minimum strength in dearth period.

12.6.1 Nectar Composition

Nectar is a solution and total solids in nectar are mostly sugars. There are only few exceptions where lipids are present in nectar. Chromatographic studies help to

know the sugars in nectar. Monosaccharides, glucose, fructose and disaccharide, sucrose, are the common sugars in nectar. In general, flowers with tubular corolla secrete sucrose dominant nectar. In open flowers, such as Brassica, only glucose, fructose and sucrose are present. Bee's preference to nectar is also governed by the sugar balance and the nectars with equal amount of glucose, fructose and sucrose are preferred by bees. Minute amounts of other substances such as amino acids, minerals, essential oils, organic acids and other components usually comprise less than 0.03 % of total dry weight. Essential oils impart characteristic aroma to nectar and honey and bees are attracted by this aroma to flower nectar. Solid particles in the form of pollen grains, yeast cells, fungal spores and bacteria can be found in small amounts.

Attempts have been made by Indian scientists to gather information on the nectar composition of bee flora. Sucrose, fructose and glucose (28:1:1) make up to 60 % of the total solids of *Thunbergia grandiflora* nectar. They also detected small amounts of aspartic acid, alanine, glycine, serine and valine by paper chromatography. *Moringa pterigosperma* Gaertn. nectar contained 0.90 % of reducing and 11.81 % of non-reducing sugars. Wakhle analysed nectars from 4 species; nectar of *Carvia callosa* contained fructose, glucose, maltose, raffinose and an unidentified sugar; nectar of *Thelepaepale ixiocephala* had only fructose, glucose and sucrose, whereas nectars of *Schefflera roxburghii* and *Grevillia robusta* had only fructose and glucose. Only these 2 sugars were observed by Mishra in *Woodfordia floribunda* nectar. Soapnut (*Sapindus emarginatus*) nectar had 85.5 % sucrose and 7.25 % each glucose and fructose. Sihag analysed the nectars of 44 plants visited by *A. florea* and *A. dorsata*. Nectar of *Tecoma stans* and all 11 cruciferous plants, *Althea rosea*, *Prunus persica*, *Prunus domestica* and *Petunia alba* contained glucose dominated sugars and sucrose and fructose were in very small fractions. Bahadur analysed nectar of 103 plants from 100 species and revealed that 54 plants had 3 sugars, viz., sucrose (S), glucose (G) and fructose (F). In addition to these, 7 plants had 4 or more sugars, 6 having S + G + F + 1 unknown sugar, 1 having S + G + F + 2 unknown sugars. Forty plants had 2 sugars (29:S + G; 3:S + F; 8:G + F) and 2 had only one each (1:G; 1:S). In the nectar of these plants, 24 had dominant sugar as sucrose, 7 had S + G, 2 had G and 1 had G + F and there was no plants having either S + F or F dominant sugar. Six plants had balanced S + G + F sugars, 3 plants had S + G and there was no plants having G + F balanced sugars. In 90 plants, amino acid was present, whereas in 15 plants the amount was double than the remaining ones.

12.7 Factors Affecting Nectar Secretion

Honey flow from the same plant is not the same under varying weather, soil and vegetation habitat conditions. The nectar secretion in a plant is the function of specific features of the forage and other external factors. Factors related to a plant species are age of the flowers and the cultivar or varieties. In *Woodfordia floribunda* Salisb, the flowers continued to secrete nectar for 3 days; it was maximum on second day and minimum on first day of flower opening. The differences in nectar secretion have also

been found in many plant species and differences with age of the flowers and cultivars have been reported in peach and cauliflower in India. Nectar secretion in flowers after opening is expected to be correlated with the degree of receptibility of the stigma. Fertilization in flowers is also known to activate a feedback mechanism to switch off nectar secretion. After certain period of flower opening, there is reduction in amount of nectar sugars. This happens because of reabsorption of nectar. Reabsorption only of nectar sugars takes place and not of water. Therefore, the reabsorption at the end of nectar secretion leads to lowered nectar sugar concentration. The total amount of nectar secretion over a period by a flower is more when it is periodically removed than in case of its non-removal by insects. Besides these, growth regulators have also been found to affect nectar volume and nectar sugars. The effect of GA₃ was more pronounced in mustard and cauliflower than with other growth regulators.

12.7.1 Sunlight and Temperature

Sunlight has a direct bearing on photosynthesis. Photosynthesis produces carbohydrates, which are secreted in nectar. Effect of sunlight and consequently of photosynthesis may not be immediate because stored carbohydrates do make good but ultimate effect is there. Temperature has direct relationship with nectar secretion. For every plant species, there is a specific threshold temperature at which the nectar secretion is started and it increases when the temperature is optimum. This is the range of temperature at which enzymes responsible for nectar secretion are activated. On the other hand, very high air temperature may result into water stress in plants and the water stress causes more water loss than the uptake. The imbalance results into lowered nectar secretion through reduced sugar transport in the conducting tissues.

12.7.2 Relative Humidity

Nectar is hygroscopic in nature and for this reason atmospheric humidity is inversely linked to nectar sugar concentration. After secretion, the nectar sugar concentration changes and attains equilibrium with the moisture in the air. Water is always lost from nectar unless the relative humidity is near 100 %. High relative humidity causing reduction in sugar concentration can affect the attractiveness of the source to bees. Conversely higher sugar concentration at lower relative humidity can affect the nectar column in the tubular flowers and bees may be unable to reach and imbibe the nectar. Water loss in the plant is also a function of relative humidity as also of air temperature.

12.7.3 Soil

Optimum soil moisture is essential for good plant growth. With soil water as a limiting factor, the number of flowers is reduced and nectar secretion is also adversely

Table 12.4 Area (million hectares) under different crops in India providing bee forage. (Anonymous 1973)

Crop	Area	Crop	Area
Jowar	14.5	Coffee	0.2
Bajr	10.4	Fruit crops	1.7
Maize	6.0	Vegetable crops	1.6
Gram	7.4	Rubber	0.5
Other pulses	17.0	Cardamom	0.1
Rapeseed, mustards	5.7	Tea	0.4
Other oil-seeds excluding groundnut	10.0	Citrus fruits and guava	0.1
Cotton	7.4	Coconut	108.0
Jute	0.8	Areca nut	1.8
Mesta	0.2	Banana	0.2

affected. Balanced nutrient level in soil which supports good plant growth also favours nectar production. Phosphorus and potassium increase nectar production but high level of phosphorus reduces it. A balance between the 2 elements should be beneficial. Excessive application of nitrogen causes abnormal vegetative growth and comparatively lesser number of flowers is produced. Some of the bee forages have been briefly discussed in this chapter.

12.8 Bee Flora of India

In several countries, beekeeping is agriculture based. Clovers, orange and other citrus fruit trees, pomaceous fruit trees, sunflower, rapeseed, mustard and other cultivated crop plants are important as sources of nectar. Honey is also produced in plantation areas with trees like locust or forests of eucalypts. In the coniferous forests of Europe, bees make honey from the sweet excretions of aphids that infest coniferous trees. This is called honeydew honey. In India, beekeeping with the Indian honey bee has been by and large forest based. In recent years, cultivated areas are increasingly used for honey production (Table 12.4, 12.5). A little over 70 % of this was under food grains including rice, wheat, jowar, bajra, maize, gram and other pulse crops. Over 24 million hectares were under oil-seed crops like ground nut and mustards. Details of the areas at present under several other cultivated plant species are not readily available. Among such species are coconut, areca nut, red oil palm, date palm, cacao, mango, custard apple, jujube, cinnamon, clove, cashew, fodder legumes, coriander, cumin, dill seed, fennel, fenugreek, garlic, turmeric, ginger and other spice and condiment crops, road side plantations that contribute to honey production like eucalyptus, karanj, tamarind, gulmohr, peltaphorum and soap nut. Hedges and fence plants like mehndi (Indian privet), duranta, mulberry, justicia and jatrophia, do also add to the bee forage value of farms and orchards (Fig. 12.1, 12.2, 12.3, 12.4, 12.5, 12.6, 12.7, 12.8, 12.9 and 12.10).

Cereals in general are not very useful as sources of bee forage. However, jowar, bajra, and maize are valued for their pollen, particularly during the kharif and rabi

Table 12.5 Bee flora of India

SN.	Scientific name	Common name	Flowering period	Source	States where found/grown	References
1	<i>Acacia</i> sp.	Acacia	5–7	NP	Punjab, Karnataka	Atwal et al. (1970), Diwan and Rao (1969) Deodikar (1970)
2	<i>Actinodaphne angustifolia</i>	Pisa	10–3	N ₁	Jammu and Kashmir	Phadke (1962)
3	<i>A. hookeri</i> Meissn	Pisa	10–1	N ₁	Maharashtra (West Coast), Karnataka	Thakar et al. (1962), Chaubal and Deodikar (1965)
4	<i>Adhatoda vasica</i> Nees	Maker	8–10	N ₃ P ₁	Maharashtra	Chaubal and Kotmire (1980), Garg (1989)
5	<i>Aegiceras corniculatum</i> (L.) Blanco	Mangrove	3–4	N ₃ P ₃	Maharashtra (Sagarmal), Himachal Pradesh, Uttar Pradesh	Chakrabarti and Chaudhary (1972)
6	<i>Albizia stipulata</i> Boivin	Ooe	4–5	N	West Bengal	Sharma and Raj (1985), Garg (1989)
7	<i>A. lebeck</i> Linn	Siris	4–5	NP	Himachal Pradesh	Sharma and Raj (1985)
8	<i>Allium cepa</i> L.	Onion	6	NP	Uttar Pradesh (Jeolikote)	Verma (1983)
9	<i>Antigonon leptopus</i> Hook & Arn.	Kaju	12–1	N ₁	Kerala, Karnataka, Orissa	Devadason (1971), Holmes
10	<i>Azadirachta indica</i> A. Juss	Coral creeper	3–4	N ₁ P ₁	Bihar	Naim and Phadke (1976)
11	<i>Bauhinia vahli</i> wt. Arn	Neem	5–6	N ₁	Uttar Pradesh	Kapil (1957), Kohli (1958)
12	<i>Berberis lycium</i> Royale	Taur	3–4	N ₁	Himachal Pradesh	Sharma and Raj (1985)
13	<i>Bidens pilosa</i> L.	Kashmal	6–10	NP	Himachal Pradesh	Sharma and Gupta (1993), Thakur and Gupta (1987)
14	<i>Bidens</i> sp.	Spanish needle	1–12	NP	Himachal Pradesh, Tamil Nadu	Sharma and Gupta (1993), Chandran and Shah (1974)
15	<i>Bombax ceiba</i> L.	–	1–13	N ₁ P ₃	Kerala	Nehru et al. (1984)
16	<i>Brassica campestris</i> L. var. sarson	Simbal	10–11	N ₁ P ₂	Bihar, Maharashtra, Uttar Pradesh, Karnataka, Himachal Pradesh	Naim and Phadke (1976), Ramesh (1980), Anonymous (1959), Sharma and Gupta (1993), Parmar (1995)
17	<i>B. campestris</i> L. var. toria	Sarson	10–11	NP	Uttar Pradesh, Bihar West Bengal, Assam, Himachal Pradesh	Kapil (1957), Parmar (1995)

Table 12.5 (continued)

SN.	Scientific name	Common name	Flowering period	Source	States where found/grown	References
18	<i>B. Compestris</i> L. var. <i>toria</i>	Toria	11-12	N ₁ P ₁	Punjab, Haryana, Himachal Pradesh	Sharma and Raj (1985)
19	<i>B. juncea</i> L. Cosson	Raya	12-2 3-4	N ₂ N ₂	Punjab, Haryana	Chaudhari (1977), Sharma (1958a)
20	<i>B. nigro</i> L. Koch	Lahi (Kali Sarson)	9-3 11	P ₂ N ₁	Jammu& Kashmir Maharashtra Uttar Pradesh	Chaubal and Kotmire (1980), Kohli (1958), Ramesh (1980), Rawat (1980)
21	<i>B. oleracea</i> L. var. <i>botrytis</i>	Cauliflower	3-5 1-2	NP NP	Himachal Pradesh Punjab	Sharma and Gupta (1993)
22	<i>Butea monosperma</i> (Lam) Taub	Dhak	1-2	N ₁ P ₁	Tamil Nadu	Khan (1948)
23	<i>Cajanus cajan</i> (L.) millsp	Arhar	9-12	N ₁	Bihar, Uttar Pradesh, Haryana, Punjab	Naim and Phadke (1976), Srawan and Sohi (1985)
24	<i>Callistemon Lanceolatus</i> DC	Bottle brush	7-8	N	Uttar Pradesh, Punjab, Himachal Pradesh, Assam, Karnataka, Maharashtra	Bhat et al. (1987)
25	<i>Carvia callosa</i> (Nees) Brem	Karvi	8-10 7-9	N ₁ P ₁ N ₁ P ₁	Maharashtra Karnataka	Phadke (1964), Chaubal and Deodikar (1965), Diwan et al. (1964), Diwan and Rao (1972)
26	<i>Camnabis sativa</i> L.	Bhang	7-9	P	Himachal Pradesh	Sharma and Raj (1972)
27	<i>Carissa spinarum</i> Linn	Garna	4-5	N ₁	Himachal Pradesh	Sharma and Gupta (1993)
28	<i>Cassia fistula</i> Linn	Amaltas	5-6	N ₂ P ₂	Himachal Pradesh	Sharma and Raj (1985), Parmar (1995)
29	<i>Cicer arietinum</i> Linn	Channa	12-2 12-2 2	N ₂ P ₂ NP NP	Maharashtra Haryana Uttar Pradesh	Chaubal and Kotmire (1980), Vasu (1967), Chaturvedi (1969), Chaudhary and Singh (1989), Srawan and Sohi (1985)
30	<i>Citrus aurantifolia</i>	Malta	12-1	N ₂ P ₂	Punjab	Chaubal and Kotmire (1980), Srawan and Sohi (1985)
31	<i>C. reticulata</i> Blanco	Mausami	3	N ₁ P ₁ N ₂ P ₂	Himachal Pradesh Kerala	Srawanj and Sohi (1984) Nehru et al. (1984)

Table 12.5 (continued)

SN.	Scientific name	Common name	Flowering period	Source	States where found/grown	References
32	<i>C. sinensis</i> (L.) Osb.	Santra/Orange	3	NP	Punjab	Srawan and Sohi (1985)
33	<i>Cocos nucifera</i> L.	Coconut	1–12	NP ₁	Karnataka, Orissa Andhra Pradesh	Anonymous (1959)
34	<i>Coffea arabica</i> L.	Coffee	4	N	Karnataka	Ramani and Bhumannavar (1990)
35	<i>Coriandrum sativum</i> L.	Dhania	1–3	N ₂ P ₂	Maharashtra, Punjab	Chaubal and Kotmire (1980)
36	<i>Dalbergia sissoo</i> DC.	Shisham	3–4	N ₁	Bihar Uttar Pradesh Himachal Pradesh, Punjab, Haryana	Chaudhrai (1977), Rehman and Singh (1940, 1941), Ramesh (1980), Parmar (1995)
37	<i>Ehretia acuminata</i> R. Br.	Punna	4	N ₁	Himachal Pradesh	Rehman and Singh (1940)
38	<i>Elaeagnus umbellata</i> Thumb	Wild olive	4–5	N ₂ P ₃	Punjab, Uttar Pradesh	Singh (1962), Chaudhari (1977)
39	<i>Eruca sativa</i> Mill	Taramira	1–5	N ₁ N ₁ P ₁	Himachal Pradesh, Punjab, Haryana, Himachal Pradesh, Uttar Pradesh	Gupta et al. (1986)
40	<i>Erythrina myosorensis</i>	Corel tree	5–6	N ₁ P ₁	Tamil Nadu	Chandran and Singh (1974)
41	<i>Eucalyptus</i> spp.	Safeda	11–12, 2–3	N ₁ P	Haryana, Punjab, Uttar Pradesh, Bihar, Karnataka, Himachal Pradesh	Srawan and Sohi (1985), Sharma and Gupta (1993)
42	<i>Eugenis</i> spp.	Bhedas, Gudda Panneralu	2–4	N ₁ N ₁	Himachal Pradesh Karnataka Kerala	Diwan et al. (1964)
43	<i>Fagopyrum esculentum</i> Moench.	Buck wheat	7–9	N ₁	Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh	Anonymous (1959) Saraf (1973)
44	<i>Fragaria vesica</i> L.	Strawberry	5–9	N	Uttar Pradesh (Jeolikote)	Singh (1979)
45	<i>Gossypium</i> spp.	Cotton	7	N P	Punjab, Haryana, Uttar Pradesh	Srawan and Sohi (1985)
46	<i>Grewia</i> sp.	Beol	5–7	N ₁ P ₁ N ₁	Karnataka Jammu & Kashmir	Diwan and Rao (1969) Deodikar (1970)
47	<i>Guizotia abyssinica</i> Cass	Niger	9–10	N ₃ P ₃	Maharashtra, Andhra Pradesh, Karnataka	Chaubal and kotmire (1980)

Table 12.5 (continued)

SN.	Scientific name	Common name	Flowering period	Source	States where found/grown	References
48	<i>Helianthus annuus</i> L.	Sunflower	7-8	N ₁ P ₃ N ₁ P ₃	Maharashtra, Punjab, Haryana	Chaubal and Kotmire (1980)
49	<i>Hevea brasiliensis</i> Muell. Arg.	Rubber	3	N ₁	Karnataka, Kerala	Srawan and Sohi (1985)
50	<i>Lagerstroemia entada</i>	Pride of India	7-9	N ₁ P ₁	Karnataka, Kerala	Diwan and Rao (1969)
51	<i>Leucas aspera</i> Link	Tumba	10-11	N ₁	Kerala	Holmes (1965)
52	<i>Litchi chinensis</i> Sonner.	Litchi	3	N ₁	Punjab, Uttar Pradesh, Bihar, Himachal Pradesh	Chaudhari (1977) Nair (1981), Kohli (1958), Chaturvedi (1969), Naim and Phadke (1976)
53	<i>Litsea stocksii</i> Hook. f	Chirandi	9-10	N ₁ P ₁	Maharashtra, Himachal Pradesh	Deodikar and Thakar (1953), Sharma and Raj (1985)
54	<i>Machilus</i> spp.	Kardel	2	N ₁ P ₁	Tamil Nadu, Assam, Himachal Pradesh	Chandran and Shah (1974), Sharma and Raj (1985)
55	<i>Madhuca longifolia</i> (Koenig), J.F. Macbr.	Mahua	2-3	N ₁ P	Bihar, Uttar Pradesh, Karnataka	Naim and Phadke (1976), Chaudhary and Singh (1989)
56	<i>Malus domestica</i> Borkh.	Apple	3-4	NP	Himachal Pradesh, Uttar Pradesh, Jammu and Kashmir	Singh (1962)
57	<i>Mangifer sativa</i> L.	Mango	1-4	N ₁	Karnataka, Kerala, Himachal Pradesh	Anonymous (1959), Sharma and Raj (1985)
58	<i>Medicago sativa</i> L.	Lucern	8-9	N ₂ P ₃	Maharashtra, Punjab	Chaubal and Kotmire (1980), Atwal et al. (1970), Chaudhari (1977)
59	<i>Melilotus</i> sp.	Clover/Melilot	3-7	N ₁ P ₁	Punjab	Chaudhari (1977)
60	<i>Mimulus elengi</i> L.	Moulsari	5-6	N ₁ P NP	Bihar Maharashtra, Orissa Assam	Naim and Phadke (1976) Subramaniam (1979)
61	<i>Moringa pterigisperma</i> Gaetrn.	Drum stick tree	1-3	NP	Bihar	Nair and Singh (1974)
62	<i>Plectranthus rugosus</i> Wall-Benth	Shain	8-10	N ₁ P ₃	Himachal Pradesh	Gupta et al. (1984b)
63	<i>Polygonum</i> sp.	Polygonum	6-9	N ₁ P ₁	Punjab	Chaudhari (1977)

Table 12.5 (continued)

SN.	Scientific name	Common name	Flowering period	Source	States where found/grown	References
64	<i>Pongamia pinnata</i> (L.) Pierre	Karanj	3	N ₁ P ₁ N ₁ P ₁ N ₃ P ₃ N ₁ P	Bihar Maharashtra Karnataka, Kerala, Uttar Pradesh, Orissa, Haryana	Naim and Phadke (1976) Chaubal and Kotmire (1980) Anonymous (1959)
65	<i>Prunus amygdalus</i> Batsch	Almond	2-3	NP	Himachal Pradesh	Sharma and Gupta (1993)
66	<i>P. armeniaca</i> L.	Apricot	2-3	NP	Himachal Pradesh	Sharma and Gupta (1993)
67	<i>P. domestica</i> L.	Plum	3	NP	Himachal Pradesh,	Sharma and Gupta (1993), Chandran and Shah (1974)
68	<i>P. pudum</i> Roxb.	Pajja	11-12	N ₁ P ₁	Tamil Nadu Himachal Pradesh	Gupta et al. (1990a), Reddy and Gupta (1987)
69	<i>Psidium guajava</i> L.	Guava	3-4	N ₂ P ₂	Maharashtra,	Chaubal and Kotmire (1980), Garg (1989)
70	<i>Pyrus communis</i> L.	Pear	2-3	NP	Himachal Pradesh Himachal Pradesh, Tamil Nadu	Sharma and Gupta (1993), Chandran and Shah (1974)
71	<i>P. pashia</i> Buch Ham	Kainth	2-3	NP	Himachal Pradesh	Sharma and Gupta (1993)
72	<i>P. persica</i> L.	Peach	2-3	NP	Himachal Pradesh	Sharma and Gupta (1993)
73	<i>Robinia pseudacacia</i> L.	Robinia/kikar	3-4	NP	Punjab Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh	Sharma and Sohi (1985) Saraf (1973), Shah (1972), Shah and Shah (1976) Gupta et al. (1992)
74	<i>Rosa moschata</i> Mill	Wild Rose	4-6	N	Himachal Pradesh	Sharma and Raj (1985)
75	<i>Rubus ellipticus</i> Smith	Akhan	1-3	N ₁	Himachal Pradesh, Uttar Pradesh, Jammu and Kashmir	Gupta and Thakur (1987)
76	<i>Rumex</i> sp.	Rumex	5-6	N	Jammu and Kashmir	Deodikar (1970)
77	<i>Salix aegyptica</i>	Willow	3-4	NP	Kashmir	Shah (1978)
78	<i>Sapindus detergens</i> Roxb.	Ritha	5	N ₁ P	Andhra Pradesh, Karnataka, Orissa, Tamil Nadu	Sharma and Raj (1985)
79	<i>S. emarginatus</i> Vahl	Ritha	10-12	N ₁ P ₃	Karnataka, Kerala	Krishnaswamy (1970)
80	<i>S. lauripolius</i> Vahl	Nauraykai	10-1	N ₁ P	Karnataka kerala	Anonymous (1959)
81	<i>S. Mukorossi</i> Gaertn.	Ritha	4	N ₁	Himachal Pradesh	Singh (1962), Goyal (1974), Sharma (1958c)

Table 12.5 (continued)

SN.	Scientific name	Common name	Flowering period	Source	States where found/grown	References
82	<i>Schefflera Wallichiana</i> Harms	Doddabettu/Po Ngabettu	5	N ₁ P ₃	Karnataka	Diwan and Rao (1972)
83	<i>Sesamum indicum</i> L.	Gingelly/Til	8-9	N ₁ P ₃	Bihar	Naim and Phadke (1976)
84	<i>Syzgium Aromaticum</i> (L.) Merrill & Jamun perry	Jamun	4-5	NP	Karnataka, Kerala	Anonymous (1959)
85	<i>Syzgium cumini</i> (L.) Skeels	Jamun	5	N ₁ P	Himachal pradesh	Sharma and Raj (1985),
			5	N ₁ P ₂		Satyarayana (1975), Nehru
			2-4	N ₁ P ₁		et al. (1984), Naim and Phadke
				N ₁ P ₃		(1976), Anonymous (1959),
				N ₁ P ₁		Chandran and Shah (1974),
				N ₁ P ₂		Chaudhary and Singh (1989),
				N ₁ P ₃		Chaubal and Kotmire (1980),
						Chaudhary (1977)
86	<i>Tamarindus indica</i> L.	Tamarind/Imli	5-8	N ₁ P	Karnataka, Kerala	Anonymous (1959), Ramachan-
				N ₁		dran (1973)
87	<i>Terminalia arjuna</i> (Roxb.) Wight & Arn.	Arjun tree	4-6	N ₁ P	Karnataka, Kerala, Uttar Pradesh, Maharashtra, Assam, Punjab	Anonymous (1959), kohli (1958), Chaubal and Kotmirew (1980), Atwal et al. (1970)
88	<i>Terminalia bellerica</i> (Gaertn.) Roxb.	Behda/Thari	4-5	N ₁ P	Karnataka, Kerala	Anonymous (1959)
89	<i>Terminalia Chebula</i> Retz.	Hirda/inknut	4-5	N ₁	Karnataka, Kerala	Anonymous (1959), Chaubal
			5-6	N ₁	Maharashtra	and Kotmire (1980), Sharma
					Himachal Pradesh	and Raj (1985)
90	<i>Terminalia tomentosa</i> Wight & Arn.	Mathi	4-6	N ₁	Karnataka	Diwan et al. (1964),
				N ₁ P	Kerala	Anonymous (1959), Thakur
				N ₃ P	Maharashtra	(1976)
91	<i>Thelepaepale Ixiocephala</i> (Benth) Bremk	Darmori/Whayati	11-1	N ₁ P ₁	Western ghats	Phadke (1965)

Table 12.5 (continued)

SN.	Scientific name	Common name	Flowering pe- riod	Source	States where found/grown	References
92	<i>Toona Ciliata</i> M. Roem	Tun	4	N ₁ N ₁ N ₁ P N ₁ P ₃ N ₁ P ₁ N ₁ P ₁	Himachal Pradesh Uttar Pradesh Jammu and Kashmir Punjab Tamil Nadu Bihar, Punjab, Haryana	Goyal (1974), Gupta et al. (1990b), Ramesh (1980), Saraf (1973), Chaudhari (1977)
93	<i>Trifolium alexandrinum</i> L.	Berseem	4–5			Chandran and Shah (1974), Naim and Phadke (1976), Srawan and Sohi (1985), Shah (1953), Chaudhari (1977)
94	<i>Vernonia</i> Sp.	Iron weed	6–9	N ₁	Karnataka	Satyanarayana (1975)
95	<i>Vitex negundo</i> L.	Banah	6–7	N	Himachal Pradesh, Uttar Pradesh	Garg (1989), Gupta and Thakur (1987)
96	<i>Wendlandia notoniana</i> Wall.	Renda/Tiliya	2	N ₁ P ₁ N ₃ P ₃	Karnataka, Maharashtra	Diwan et al. (1964)
97	<i>Woodfordia floribunda</i> Salisb.	Dhai	4–5	N	Himachal Pradesh	Thakur et al. (1962), Mishra et al. (1987)
98	<i>Ziziphus mauritiana</i> Lam.	Ber	7–10	N ₃ P ₃	Maharashtra, Haryana, Orissa, Uttar Pradesh	Chaubal and Kotmire (1980), Chaudhary and Singh (1989)

N nectar, *P* pollen, *N*₁ major honey source, *P*₁ major pollen source, *N*₂ medium honey source, *P*₂ medium pollen source, *N*₃ minor honey source, *P*₃ minor pollen source, 1–12 months of the calendar year, January–December



Fig. 12.1 Forage plants of *A. cerana* (wild flowers). *Hyptis suaveolens*, *Hamelia patens*, *Portulaca oleracea*, *Justicia procumbens*, *Antigonon leptopus*, *Duranta repens*, *Sterculia foetida*, *Spathodea campanulata*, *Sapindus emarginatus*, *Pterocarpus santalinus*, *Pongamia pinnata*, *Helicteres isora*, *Pithecellobium dulce*, *Diospyros chloroxylon*, *Terminalia tomentosa*, *Gliricidia sepium*, *Mimosa pudica*, *Eupatorium odoratum*, *Moringa oleifera*, *Argemone mexicana*, *Jatropha pandurifolia*, *Cocos nucifera*, *Croton bonplandianum*, *Tridax procumbens*, *Solanum melongena*, *Malvastrum coromandelianum*, *Rhynchosia beddomei*, *Evolvulus alsinoides*, *Datura metel*, *Vitex altissima*



Fig. 12.1 (continued)



Fig. 12.2 Forage plants of *A. cerana* (ornamental flowers)

crop seasons when natural sources are scarce. In the case of jowar, some varieties produce sugary exudation on the nodes. Leaves also secrete a thick sweet liquid when infected by rust fungi. In both cases, bees collect the sweet substances. Among other crops which are not useful to honey bees are ground nut and sugar cane. The latter is occasionally useful as a supplementary source of carbohydrate food. This happens after the crop is harvested. Honey bees eagerly visit the freshly cut stumps for the sugary sap, oozing out of them.

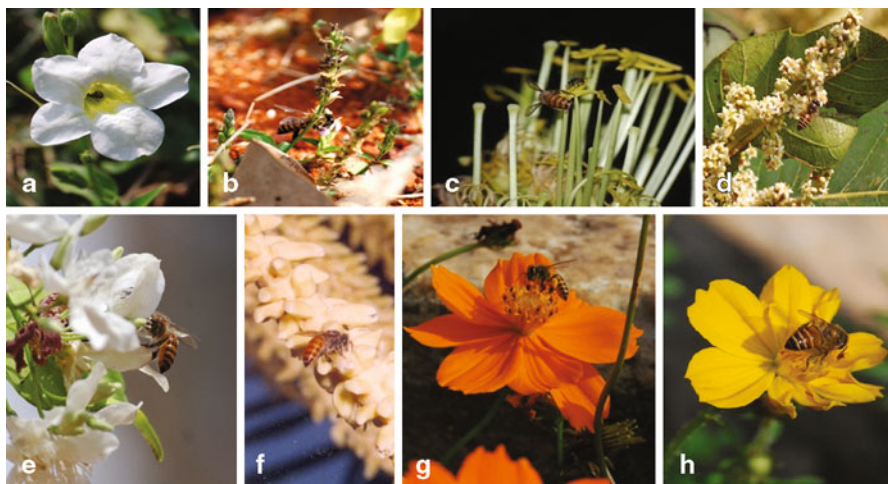


Fig. 12.3 Forage plants of *A. cerana* (miscellaneous flowers). Forage plants of *A. cerana*. **a** *Asystasia gangetica*. **b** *Justicia procumbens*. **c** *Agave americana*. **d** *Buchanania lanzan*. **e** *Wrightia tinctoria*. **f** *Cocos nucifera*. **g** *Chromolaena odorata*. **h** *Cosmos bipinnatus*. **i** *Cosmos sulphureus*

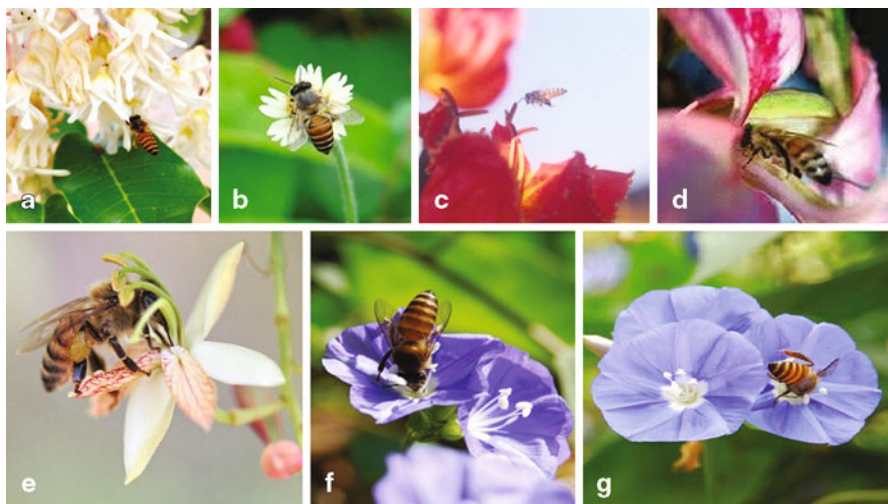


Fig. 12.4 Forage plants of *A. cerana* (miscellaneous flowers). **a** *Tridax procumbens*. **b** *Spathodea campanulata*. **c** *Bauhinia purpurea*. **d** *Tamarindus indica*. **e**, **f** *Evolvulus alsinoides*. **g** *Ipomoea pes-caprae*. **h** *Shorea roxburghii*

Many pulse and oil-seed crops are good sources of bee forage. Among the plantation commercial crops, coffee, orange and other citrus fruit, apple and other pomaceous fruit species, cardamom and rubber tree are important from the bee-keeping point of view. The last mentioned is the single most largest source of nectar



Fig. 12.5 Forage plants of *A. cerana* (miscellaneous flowers). **a** *Diospyros chloroxyylon*. **b** *Croton bonplandianum*. **c** *Jatropha pandurifolia*. **d** *Gliricidium sepium*. **e** *Pongamia pinnata*. **f** *Pterocarpus santalinus*. **g** *Rhynchosia beddomei*. **h** *Hyptis suaveolens*. *Crocus sativa*

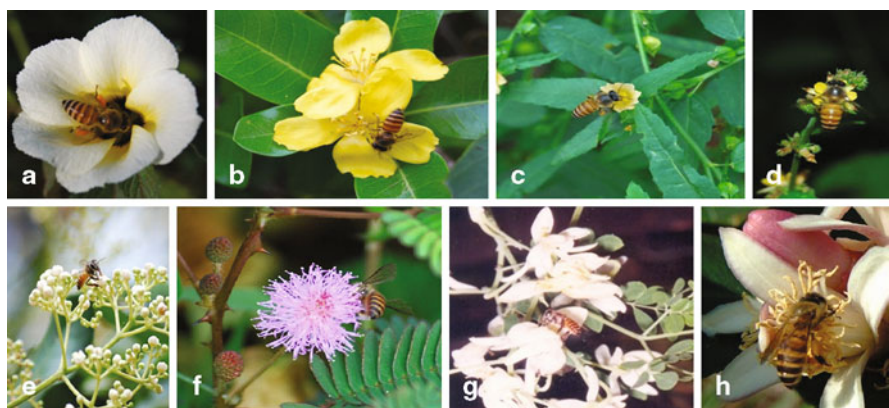


Fig. 12.6 Forage plants of *A. cerana* (miscellaneous flowers). **a** *Hugonia mystax*. **b** *Malvastrum coromandelicanum*. **c** *Sida acuta*. **d** *Triumfetta pentandra*. **e** *Walsura trifoliata*. **f** *Mimosa pudica*. **g** *Pithecellobium dulce*. **h** *Moringa oleifera*. *Citrus reticulata*

in India. Rubber plantations are found in southwestern and northeastern parts of India, where tropical humid climate prevails. Kerala, Tamilnadu, Karnataka, and Tripura have large areas under rubber plantation. The nectaries on young leaves of rubber trees secrete nectar profusely in the refoliation stage, before the tree blooms. Next in importance is litchi tree. The entire north India from West Bengal to Jammu has large areas under litchi orchards that constitute an excellent source of nectar during March and May. A point to note here is the relatively low utility of garden plants. Several garden annuals, shrubs and climbers are not useful to honey bees, mainly because the showy flowers lost their reproductive function and produce no

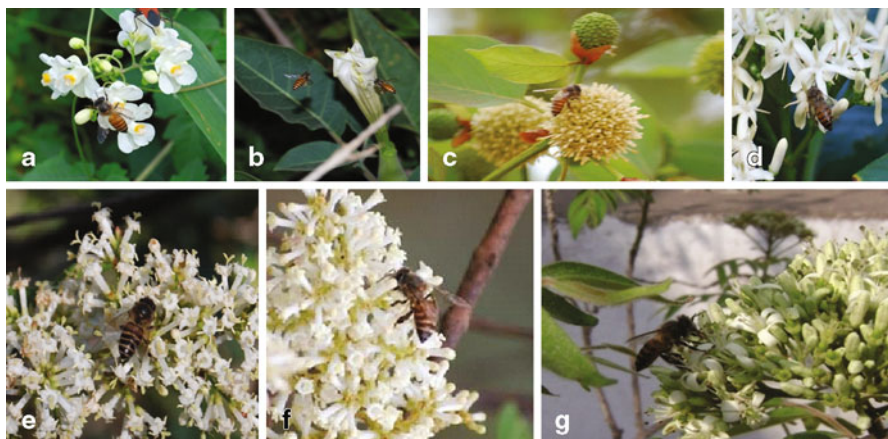


Fig. 12.7 Forage plants of *A. cerana* (miscellaneous flowers). **a** *Mitragnya parviflora*. **b** *Tarenna asiatica*. **c** *Wendlandia glabrata*. **d** *Wendlandia tinctoria*. **e** *Murraya koenigii*. **f** *Cardiospermum canescens*. **g** *Sapindus emarginatus*. **h** *Datura metel*

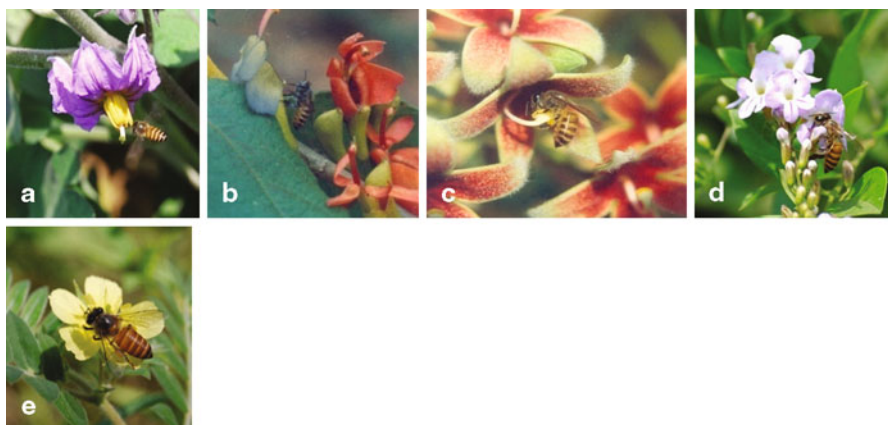


Fig. 12.8 Forage plants of *A. cerana* (miscellaneous flowers). **a** *Solanum melongena*. **b** *Helicteres isora*. **c** *Sterculia foetida*. **d** *Durlanta repens*. **e** *Vitex altissima*. **f** *Tribulus terrestris*

pollen or nectar. Some garden species do however contribute to the bee forage in the otherwise useless areas under gardens and parks. These include the railway creeper, ocimums, salvias, coleus, poinsettia, petunia, zinnia, phlox and daisy.

12.8.1 Avenue, Amenity and Timber Tree

Eucalyptus spp. (Fam.: Myrtaceae). These are evergreen trees and are indigenous to Australia. In Australia, most species are bushes and shrubs but fortunately in

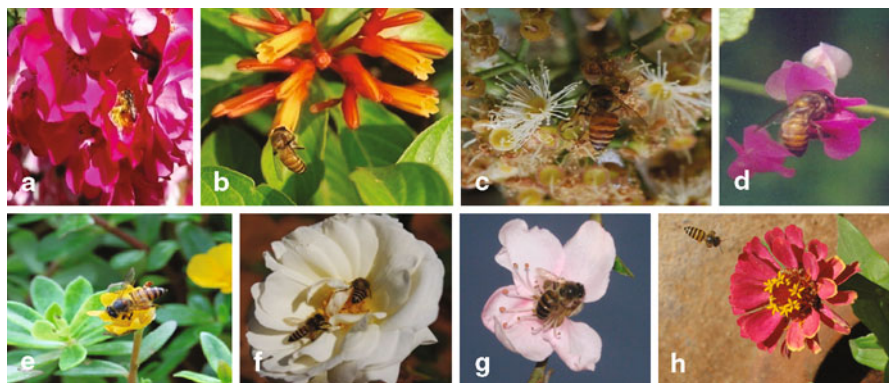


Fig. 12.9 Forage plants of *A. cerana* (miscellaneous flowers). **a.** *Syzygium alternifolium*. **b.** *Syzygium cumini*. **c.** *Antigonon leptopus*. **d.** *Portulaca oleracea*. **e.** *Rosa centifolia*. **f.** *Rosa* sp. **g.** *Borreria hispida*. **h.** *Hamelia paten*. **i.** *Prunus persica*. ornamental flowers of composite spp

India *Eucalyptus* spp. attain gigantic size. Different species and even varieties flower during different parts of the year but main bloom is available in spring. *Eucalyptus* plantations have expanded very fast on road sides, canals, even waste lands and thus presenting vast potentials to bees. Honey is light amber coloured and granulation is fairly quick.

Hirad (*Terminalia chebula* Retz; Fam.: Combretaceae). Hirad is distributed in the Western Ghats and in submountainous regions. The fruits have medicinal value and also a source of dyes and tannins. It is a major flora in beekeeping areas of Maharashtra. It flowers in April–June. Hirad honey is composed of 17 % water, 35 % glucose, 40–41 % fructose, sucrose, maltose, melezitose, ash and it also contains calcium, phosphorus, iron, magnesium, sodium, potassium and silicon. Tartaric, citric, malic, succinic acids are also present. Honey is light yellow coloured; granulation is slow and has characteristic pungent aroma and tastes akin to fruit tannins. *Terminalia arjuna* (Rob.) Wight and Arn. has been widely planted in forests and as avenue plantation. It is also a nectar and pollen source but details of value as bee forage have not been worked out. *T. bellerica* (Gaertn.) Roxb. has also both nectar and pollen forage but no data on nectar and honey is available.

Jamun (*Syzygium cumini* Skeels; Fam.: Myrtaceae). Jamun is widely planted avenue-cum-fruit tree and naturalized as forests in many parts of India. It is a predominant flora of Western Ghats. It is reported to be a major flora in Bihar, Maharashtra, Punjab, Tamil Nadu and Uttar Pradesh. Its flowers are dirty white and bloom in April–May. The honey flow extends over a period of 2 or 3 weeks. Nectar-sugar concentration is very high, up to 72 % but may be as low as 9 %. Jamun is also a pollen source. Chemical composition of honey has been given by Narayana. Fructose is high (43.30 %) and glucose is 32.26 %, sucrose, maltose, reffinose and melezitose are present in jamun honey. Protein (0.656 %), dextrin (1.55 %), ash (0.182 %) and riboflavin, ascorbic acid, thiamine and niacin are also



Fig. 12.10 Forage plants of *A. cerana* (miscellaneous flowers). **a** Citrus spp. **b** Strawberry. **c** Sarson. **d** Chrysanthemum. **e** Cauliflower. **f** Portulaca. **g** Rosa spp. **h** Cauliflower. **i** *Bignonia venesta*. **j** *Callistemon lanceolatus*. **k** Pomegranate. **l** Unidentified. **m** Cauliflower. **n** *Vitex negundo*

present in honey. It is light reddish-brown and has characteristic taste of jamun fruit. The honey does not granulate for years.

Karanj (*Pongamia pinnata* (L. Pierre); Fam.: Leguminosae). Karanj grows well in humid tropical area. *P. glabra* is grown as avenue and shade tree in drier climate. Leaves are used as fodder. It blooms in April–May and both nectar and pollen are available from karanj. Data on nectar production, honey characteristics and honey potentials are not available but it is considered to be good flora by the beekeepers.

Moulsari (*Mimusops elegni* L.; Fam: Sapotaceae). Moulsari is evergreen tree with whitish flowers. It has good nectar flow in May–June in Bihar and also in Uttar Pradesh. Bees also collect pollen from this flora. No information on nectar and honey from moulsari is available.

Phalsa (*Grewia asiatica* L.; Fam.; Tiliaceae). Phalsa shrub is grown for its fruits. It blooms from April to August and yields nectar to bees. The extent of its cultivation is less. *G. oppositifolia* Roxb. is a medium-sized tree which is planted for lopping its foliage for fodder, for its best fibre for ropes and for timber. It blooms in April–May and is a nectar source to bees. These species of *Grewia* are only minor sources.

Rubber (*Hevea brasiliensis* Muell. Arg.; Fam.; Euphorbiaceae). Rubber plant is deciduous tree with male and female flowers on the same inflorescence. Nectar is available to bees only from the extra-floral nectaries at the base of young buds. The nectar flow is there for about 2 weeks when leaves are young. Rubber is a major flora in Kerala where large plantations are grown. Honey potential is estimated to be 3 kg per tree. Rubber honey is clear straw coloured.

Schefflera wallichina Harms. (Fam.; Araliaceae). It grows as a strangler or small tree in forests or cardamom estates of Coorg area of Karnataka. Schefflera blooms in April–June and is a rich nectar source. Most honey in Coorg is obtained from this source. Pollen availability to bees is very low.

Shisham (*Dalbergia sissoo* DC.; Fam.; Leguminosae). Shisham's habitat is very varied, from lower hills to dry plains; normally planted on banks of canals or roadside as shade tree. This is a source of valuable timber especially for furniture. Pale-yellowish flowers are put forth in April. Corolla is tubular but the narrow tube has nectar well up to the reach of bees. Wind blows flower from branches and nectar availability is adversely affected. It is a major nectar source for bees in many states, especially Himachal Pradesh, Punjab, Haryana and Uttar Pradesh. Shisham honey is dark amber with moisture 18.75 %, glucose 34.6 %, fructose 39.1 %, sucrose 1.04 % and ash 0.18 %. Honey flavour is strong and not attractive.

Silver oak (*Grevillea robusta* A. Cunn. ex R. Br; Fam.; Proteaceae). Silver oak is planted in coffee plantations and also as shade and avenue tree. It profusely flowers and is an important nectar source and nectar secretion is abundant with 17–79 % nectar concentration. Honey is reddish-black with prominent flavour and it granulates rapidly.

Soapnut (*Sapindus* spp.; Fam.; Spindanceae). Soapnut is an avenue and forest tree; fruits are used as substitute to soap. *S. emarginatus* Vahl is a species found in Andhra Pradesh, Karnataka, Orissa and Tamil Nadu. The species flowers in October–December. It gives 20–25 % of total honey yield in some parts of Andhra Pradesh. *S. emarginatus* nectar has 85.5 % sucrose and 7.25 % glucose and fructose.

Tamarind (*Tamarindus indica* L.; Fam.; Leguminosae). Tamarind is a large evergreen shade tree cultivated in many parts of India for pods which are used in curries. The tree has many other uses like fuel, timber, etc. It blooms in April–July and is a good nectar and minor pollen source in South India. Honey is rich golden and has sour flavour.

Toon (*Toona ciliata* M. Roem.; Fam.; Meliaceae). Toon grows as timber and avenue tree in lower hills and plains of northern India especially in moist soil. It is also lopped for fodder but is not to the liking of cattle. Toon is a major nectar source in Himachal Pradesh, Kashmir, Punjab and Uttar Pradesh. It is a minor source of pollen. Large number of trees has recently been cut and floral source has declined. Nectar sugar concentration varies from 26 to 72 % in freshly opened to 48 h old flowers. Average nectar sugar value is 2.38 mg/flower/day and nectar is secreted for 4 days. Honey is light amber in colour with pronounced flavour. Normally its honey is mixed with *Dalbergia sissoo* honey and is obtained in May.

Whayati (*Thelepea ixiocephala* (Benth.) Bremk; Fam.: Acanthaceae). Whayati is a tree of moist forests of Western Ghats of Maharashtra and Karnataka. Whayati flowers after every 8 years. It blooms in November–January and is a major nectar source. Sugar concentration of nectar is 35–46 %. Honey contains 38 % glucose and 39 % fructose; sucrose, maltose, raffinose and melezitose are present. Sodium, potassium, calcium, magnesium, iron, phosphorus and silicon are contained in whayati honey. It is light yellow and granules rapidly but granulation is uniform.

There are many other avenue and forest trees which are good source of nectar and pollen but their number is small and at best serve as subsistence sources. These trees are bottle brush (*Callistemon lanceolatus* DC.), pride of India (*Lagerstroemia indica* L.), drum stick (*Moringa oleifera* Lam.), Indian laburnum (*Cassia* spp.), puna (*Ehretia acuminata* B.), siris (*Albizia* spp.), willows (*Salix* spp.) and chestnuts (*Aesculus* sp., *Castanea* sp.)

12.8.1.1 Fruits

Banana (*Musa* spp.; Fam.; Musaceae). Perennial herb, stem formed by leaf petioles. Flowers are large and monoecious. *Musa* spp. flowers throughout the year and is a medium to good source of nectar which has 25–30 % sugar concentration. Banana flowers are also visited by bees for pollen which is in abundance. Banana plantations are common in many states of India.

Cashew (*Anacardium occidentale* L.; Fam.: Anacardiaceae). Cashew is cultivated in South India. It is an evergreen tree, flowers pink, small and fragrant. Devadason has advocated migratory beekeeping to avail cashew flow. It is also a pollen source; value of the flora and honey characteristics are not known.

Citrus spp. (Fam.: Rutaceae). These are *C. aurantifolia* (Christm) Swingle; *C. grandis* (L.) Osbeck; *C. limon* (L.) Burm.; *C. paradisi* Macfad.; *C. reticulata* Blanco and *C. sinensis* (L.) Osb. These citrus species flower during February and March. Nectar production and nectar sugar concentration is medium. Citrus spp. also serve as pollen source. Citrus honey has delicate flavour. In India, Citrus mostly serves as

a build up flora and surplus is not extracted anywhere, though large areas are under citrus and it is widely distributed.

Coconut (*Cocos nucifera* L.; Fam.: Palmae). It is grown in coastal regions. Flowers are small, monoecious, both male and female flowers have nectaries. It blooms in May–June and bees collect abundant pollen from staminate flowers in spathes.

Jujube (*Ziziphus mauritiana* Lam.: Fam.; Rhamnaceae). Indian jujube is cultivated in tropical parts of India, advantageously below 600 M.S.L. (mean sea level). It tolerates severe heat and is drought resistant. It flowers during July and October and flowering is very protracted. The flora gives surplus honey when the colonies are strong but bees get nectar source when there is no other flora. Some pollen is also availed and therefore, it is very useful forage for bees. The jujube honey is yellow-brown with very sweet flavour.

Litchi (*Litchi chinensis* Sonner; Fam.: Sapindaceae). Litchi has become very popular in sub-mountainous regions for expensive fruits. It blooms in March and is a rich source of nectar to bees. Sugar concentration of nectar is high. Juice from damaged fruits is also availed by bees. Litchi honey is light golden coloured with very pleasing aroma.

Pome and stone fruits. Apple, pear, plum, peach, apricot, cherry, almond and their closely allied wild species are included in this category. They flower from February to April and bees gather both nectar and pollen. These fruit trees have local importance and have good build up sources before surplus honey flow season. Pear nectar is very low in sugar concentration. Therefore, it is normally avoided in favour of other competing flora. Surplus honey is not gathered from these cultivated fruit trees. However, the wild cherry, *Prunus puddum* Roxb. flowers in October–November when no other flora is available in mid and lower hills of Himachal Pradesh. On an average, a flower secretes 35 ml nectar for 4 days with 3.47 mg nectar sugar per flower. Nectar sugar concentration varies from 12 to 18 %. Chromatographic separation revealed glucose, fructose, sucrose and one unidentified sugar in the ratio of 39.6, 40.7, 12.3 and 7.5.

12.9 Nectar and Honey Potential

The amount of nectar produced during a season per hectare of land is called *honey potential*. A plant producing large quantity of nectar with surplus honey is referred to as *major source* and if a plant provides nectar just for survival of the colonies it is called *minor source*. As the honey is produced from nectar, the amount of honey produced per hectare of land from a plant species under optimal conditions is called as *honey potential*. Honey potential varies from species to species, with climate, soil and geographical conditions (Table 12.6, 12.7). Factors affecting nectar production such as temperature, humidity, soil moisture also influence the honey production potential of a crop.

Table 12.6 Honey production potential of some common bee plants. (Joshi 1998)

Plant species	Honey potential (kg/ha)	Remarks/factors
<i>Brassica campestris</i>	50–100	Needs good irrigation
<i>Eucalyptus spp.</i>	1000–1500	Varies with age and size of the trees
<i>Litchi chinensis</i>	500–1000	Healthy plants
<i>Dalbergia sissoo</i>	200–300	Requires good rains during winter
<i>Syzygium cumini</i>	200–300	Large healthy trees
<i>Azadirachta indica</i>	100–200	Large healthy trees
<i>Cedrella toona</i>	300–500	Uniform plantation and age of trees
<i>Sapindus mukorossi</i>	300–500	Uniform plantation and age of trees
<i>Berberis spp.</i>	100–300	Thick bushes
<i>Terminalia chebula</i>	100–300	Healthy trees and Uniform plantations
<i>Prunus armeniaca</i>	100–300	Uniform plantation and healthy trees
<i>Malus domestica</i>	15–20	Requires plentiful rains during winter
<i>Trifolium alexandrinum</i>	300–500	Requires good irrigation
<i>Gossypium spp.</i>	100–300	Healthy crop, good irrigation
<i>Robinia pseudoaccacia</i>	500–1000	Requires plenty of soil moisture
<i>Acacia catechu</i>	100–300	Requires plentiful rains before flowering and light rain during flowering
<i>Trifolium repens</i>	200–500	Requires good irrigation and soil moisture
<i>Plectranthus spp.</i>	500–1000	Requires plentiful rains during rainy season and good soil moisture
<i>Prunus cerasoides</i>	100–300	Requires good soil moisture
<i>Brassica napus</i>	300–500	Requires good irrigation
<i>Helianthus annuus</i>	100–200	Requires good irrigation

12.10 Sources of Bee Forage in India

It is believed that the highly advanced insect groups like the bees and the flowering plants evolved during the course of the last 100 million years, by influencing each other due to their inter-dependence. Plants depend on anthophilous insects for pollinating their flowers resulting in fruit and seed set. Honeybees depend upon flowering plants for their food. In any area with good vegetation, the natural or cultivated plant species are in an ecological balance with the local insect population.

There are several thousands of species flowering plants indigenous to India. In addition, there are a few thousand plant species that have been introduced as crops of economic importance, or as ornamental plants. Not all these are useful to honey bees, and even those that provide bee forage vary in their value to beekeeping. There are also variations in the utility of a species depending upon the climate, soil and other factors in the place of its occurrence. In assessing the beekeeping potential of any location it is of prime importance to learn about the composition of the vegetation in the locality, bee forage value of individual species and the usual flowering periods of important bee forage plants.

Sequence of flowering of the component plant species in the area has to be observed to find out the seasons when a large amount of nectar is available, which helps in storage of surplus honey that can be harvested by the beekeeper. This observation also helps to find out whether bee forage is continuously available for at least eight months in a year. Long gaps in the bee forage availability affects the growth of bee

Table 12.7 Bee flora, their nectar sugar concentration, sugar value and honey production potential capacity

Botanical name	Common name	Nectar sugar concentration (%)	Sugar value (mg/flower/24)	Honey production potential (kg/colony/season)	Reference
<i>Allium cepa</i>	Onion	59–75.5	–	–	Rao and Lazar 1980
<i>Bombax ceiba</i>	Simbal	35.0	–	–	Parmar 1995
<i>Brassica campestris</i> L. var <i>toria</i>	Toria	38.5–53.5	–	–	Kapil and Brar 1971
<i>Callistemon lanceolatus</i>	Bottle brush	23.2	1.21	–	Bhat et al. 1987
<i>Carvia callosa</i>	Karvi	20–37	–	–	Phadke 1962
<i>Citrus aurantifolia</i>	Malta	22.0	–	–	Srawan and Sohi 1985
<i>Dalbergia sissoo</i>	Shisham	32	–	4–9	Singh 1962
<i>Eucalyptus spp</i>	Safeda	66–67	–	–	Srawan and Sohi 1985
<i>Fagopyrum esculentum</i>	Buckwheat	35–45	0.10–2.68	60–70 70–90	Cirnu et al 1977 Avetisyan 1978
<i>Helianthus annuus</i>	Sunflower	35–38	0.27	56–69	Peter 1977
<i>Litchi chinensis</i>	Litchi	More than 62	–	27	Nair 1981
<i>Malus domestica</i>	Apple	30–65	1–3	36	Lovell 1977
<i>Medicago sativa</i>	Lucerne	40.2	1.13	–	Bhat et al. 1987
<i>Plectranthus rugosus</i>	Shain	38	–	50	Shah 1972
<i>Robinia pseudoacacia</i>	Robinia false kikar	34–59	0.76–4.0	80	Konstantinovic 1977
<i>Sapindus detergens</i>	Ritha	40	–	–	Sharma 1958a
<i>Syzygium cumini</i>	Jamun	9–72	–	–	Satyanaryana 1975
<i>Trifolium alexandrinum</i>	Berseem	32.4	–	165	Petkov 1977
<i>Vitex negundo</i>	Banah	32–42	0.39	–	Gupta and Thakur 1987
<i>Ziziphus Mauritiana</i>	Ber	More than 40	–	–	Zmarlicki 1984

colonies. During such floral dearth seasons, particularly when pollen is not available, colonies become weak. Such colonies use up a major part of the forage available immediately after the dearth for recouping their strength and build up of worker bee population. Often the important honey season that follows the dearth period is halfway through before these colonies can start storing surplus honey. Colonies can be shifted between farms and forests, depending upon the availability of bee forage or its absence in any place. Thus both the natural and cultivated vegetations are complementary for proper growth of bee colonies and for efficiency in the honey industry. Deodikar and Suryanarayana (1977) felt that the agricultural crops could give only seasonal forage for bees restricted to their flowering periods around September–October, and December–January in the case of annual summer and winter crops, and around March–April for most of the orchard and other arboreal species. In India,

the dearth periods on farms and orchards in plains more or less synchronized with floral peak periods in nearby forests in hills. Deodikar and Suryanarayana, therefore, felt that the 120 million colonies that would be required for pollinating crop plants, could be accommodated in the 60 million hectares under reserved forests.

Knowledge of bee plant species is necessary for the beekeeper so that he can make an efficient use of these resources. In view of this, there have been several observations on bee plants of different locations (Deodikar and Suryanarayana 1977). Similarly many other workers have evaluated the honey plants useful for bees (Saraf 1972; Singh and Singh 1971; Atwal and Goyal 1974; Pratap 1997). Still there may be many plants important to honey bees in India which may have been left out because of the paucity of information on them.

In both stationary and migratory beekeeping, the beekeeper seeks to place his colonies in or near areas where a sufficient quantity of honey plants—be they crop or pasture plants, weeds, shrubs, forest trees, roadside planting, etc. exists, in season or throughout the year, within the economical flight range of the foragers. Planting special crops for bees is not likely to yield a good economic return: arable land will provide better returns if it is used for other agricultural purposes. Beekeeping is thus one of the rare forms of agriculture in which the planting of crops is not specifically required.

12.10.1 Lists of the Bee Plants Recommended for Propagation in Different Climatic Zones

Deodikar and Suryanarayana (1977) have provided a very comprehensive list of bee forage plants available in different parts of India (Table 12.8). These can be planted under different climatic and edaphic conditions. Plants are listed here under different climatic regions, viz. arid or semi-arid regions, tropical humid climates, sub-tropical and temperate regions, sea coasts and marsh lands. Plants are also listed for social forestry, garden ornamentals and avenues. Common and vernacular names are given for each species, wherever possible. The language is indicated in brackets—H: Hindi; Tam: Tamil; Tel: Telugu; Kan: Kannada; Mal: Malayalam; Guj: Gujarathi; Ben: Bengali; O: Oriya; Mar: Marathi; P: Punjabi.

12.11 Nectar Potential in India

A beekeeper must know the nectar potential of his locality before making any investment to start this enterprise. Nectar is the basic raw product of honey. The days when a good number of plants have nectar to be foraged by honey bees is called a honey flow period. If the nectar yield is copious and obtained from a good number of plants of a particular species, it is called major honey flow period. When the amount of nectar to be collected is small it is called a minor flow period and the days

Table 12.8 Bee plants recommended for propagation in different climatic zones. *Arid or semi-arid regions/deciduous forests*

Sr. No.	Botanical name	Family	Common/local names	Economic use
1.	<i>Acacia auriculiformis</i> A. Cunn. Ex Benth	Mimosaceae	Austrilian Phyllode acacia	Fire wood
2.	<i>Acacia catechu</i> (Linn. F.) Willd.	Mimosaceae	Cutch tree; Khair (H)	Cutchu from heart wood
3.	<i>Acacia senegal</i> (Linn.) Willd	Mimosaceae	Kher	Gum
4.	<i>Acacia sinuata</i> (Lour.) Merr.	Mimosaceae	Soap pod wattle; Shikakai (H); Shikai (Tam)	Soapnut; bark for dyeing and tanning; live fence; firewood
5.	<i>Adenanthere pavonina</i> Linn.	Mimosaceae	Coral wood; Barighumchi (H); Rakta Kambal (Beng)	Timber
6.	<i>Aegle marmelos</i> (Linn.) Correa	Rutaceae	Bael tree; Bel (H); Vilvam (Tam); Maredu (Tel)	Fruits edible; medicinal; timber
7.	<i>Anthocephalus cadamba</i> (Roxb.) Miq.	Rubiaceae	Kadamba (H); Kadam (Beng); Vellaicadamba (Tam)	Wood useful; flowers fragrant
8.	<i>Bombax ceiba</i> Linn.	Bombacaceae	Red silk cotton; Semul (H); Lal Sawar (Mar)	Seed fibre; timber
9.	<i>Buchanania lanzan</i> Spreng	Anacardiaceae	Sara (Tel); Chironji (H); Morala (Tam)	Fruit and seeds edible; oil from kernels
10.	<i>Ceiba pentandra</i> (Linn.) Gaertn.	Bombacaceae	White silk cotton; Safed-simul Katesawar (Mar); Ilavu (Tam)	Seed fibre; timber
11.	<i>Dalbergia</i> spp.	Fabaceae	Shisham etc.	Durable timber
12.	<i>Embllica officinalis</i> Gaertn.	Euphorbiaceae	Embllic myrabolan; Amla (H); Nelli (Mal and Kan); Avla (Mar)	Fruits edible; medicinal
13.	<i>Eucalyptus intertexta</i> R.T. Baker	Myrtaceae	Red bore	Excellent charcoal
14.	<i>Flacourtia indica</i> (Burm. f.) Merr.	Flacountriaceae	Sweet thorn	Firewood
15.	<i>Gmelina arborea</i> Linn.	Verbenaceae	Ghamar (H); Kattanam (Tam)	Timber; dodder
16.	<i>Leucaena</i> spp.	Mimosaceae	Soobabul (H and Mar); Tagarai (Tam); Lasobaval (Guj)	Green manure; dodder; timber; firewood
17.	<i>Limonia acidissima</i> Linn.	Rutaceae	Wood apple; Kait (H); Velaga (Tel); Kavath (Mar)	Fruits edible; gum; wood
18.	<i>Madhuca</i> spp.	Sapotaceae	Mohwa, etc.	Timber; oil from seeds; fruits edible
19.	<i>Moringa oleifera</i> Lamk.	Moringaceae	Drumstick tree; Sainjana (H); Murangai (Tam); Shevga (Mar); Munaga (Tel)	Leaves, flowers and fruits eaten as vegetables; medicinal
20.	<i>Parkinsonia aculeata</i> Linn.	Caesalpinjiaceae	Jarusalem thorn; Vilayati Kikar (H)	Ornamental; avenue tree

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
21.	<i>Phoenix dactylifera</i> Linn.	Arecaceae	Date Palm; Pindkhajur (H)	Fruits edible; leaves for fibre
22.	<i>Pongamia pinnata</i> (Linn.) Pierre	Fabaceae	Indian Beech; Karanja (H); Pangam (Tam); Ganuga (Tel)	Non-edible oil; timber; firewood; medicinal
23.	<i>Pterocarpus marsupium</i> Roxb.	Fabaceae	Kino tree; Bijasal (H); Vengai (Tam)	Wood; red-gum resin used in medicine
24.	<i>Schleichera oleosa</i> (Lour.) Oken	Sapindaceae	Kusum (H); Kusumb (Mar)	Host for lac-insect; seed oil; young fruits edible
25.	<i>Strychnos nux-vomica</i> Linn.	Strychnaceae	Nux-Vomica; Kuchla (H); Yetti (Tam)	Wood for agricultural implements; seed yield strychnine alkaloid; medicinal
26.	<i>Tamarindus indica</i> Linn.	Caesalpiniaceae	Tamarind tree; Imli (H); Puli (Tam); Tantal (O)	Fruits and young leaves edible; condiment; timber; firewood
27.	<i>Terminalia arjuna</i> Wt. and Arn.	Combretaceae	Arjun (Mar)	Wood used for agricultural implements
28.	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	Bahera (H)	Wood
29.	<i>Vitex altissima</i> Linn. F.	Verbenaceae	Milla; Maila (Tam)	Wood
30.	<i>Wendlandia</i> spp.	Rubiaceae	Thavsa (Mar); Bankat, etc.	Wood useful; leaves for fodder
31.	<i>Ziziphus</i> spp.	Rhamnaceae	Chinese date; Ber (H); Bor (Mar); Regu (Tel)	Fruits edible; fodder; firewood
Tropical (humid) climates				
1.	<i>Acacia ducurrens</i> Wild.	Mimosaceae	Green wattle	Bark for tannin; firewood
2.	<i>Actinodaphne angustifolia</i> Nees	Lauraceae	Pisa	Non-edible oil; fuel wood
3.	<i>Alseodaphne semicarpifolia</i> Nees	Lauraceae	Phudgus (Mar); Yavaranai	Timber (grows in cooler climates)
4.	<i>Averrhoa carambola</i> Linn.	Averrhoaceae	Carambola tree	Fruits edible
5.	<i>Banksia serrata</i> Linn. F.	Proteaceae	Australian honey suckle	Wood for boat knees; bullock yokes
6.	<i>Bischofia javanica</i> Bl.	Bischofiaceae	Bishop wood; Paniala (H); Boke (Mar); Nalupumashti (Tel)	Timber
7.	<i>Canthium parviflorum</i> Lamk	Rubiaceae	Kirmi (H)	Wood used for toys; fruits edible
8.	<i>Cinnamomum zeylanicum</i> Bl.	Lauraceae	Cinnamon; Dalchini(H); Hayangam (Tam)	Bark yields spice
9.	<i>Cryptocarya wightiana</i> Thw.	Lauraceae	Palai(Tam); Gulmur(Kan) Chaltia (H and Beng);	Wood useful

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
10.	<i>Dillenia indica</i> Linn.	Dilleniaceae	Chalta (H and Beng);	Timber; jam and jellies from fruits
11.	<i>Diospyros Kaki</i> Linn. f.	Ebenaceae	Persimmon	Fruits
12.	<i>Elaeocarpus</i> spp.	Elaeocarpaceae	Rudraksham	Timber
13.	<i>Erioglossum rubiginosum</i> Bl.	Sapindaceae	Ritha	Fruits edible
14.	<i>Eucalyptus calophylla</i> R. Br. ex Lindl.	Myrtaceae	Redgum	Timber; oil from leaves
15.	<i>Euphoria longan</i> (Lour.) Steud.	Sapindaceae	Longan; Ashphal(Beng); Wumb(Mar); Puvatti(Tam)	Fruits edible; wood useful
16.	<i>Carcinia indica</i> Chois.	Guttiferae	Red Mango	Fruits edible
17.	<i>Grewia</i> spp.	Tiliaceae	Phalsa etc.	Excellent timber; fruits edible
18.	<i>Hevea brasiliensis</i> (HBK) Muell.-Arg.	Euphorbiaceae	Para rubber	Rubber from latex
19.	<i>Lagerstroemia</i> spp.	Lythraceae	Jarul etc.	Timber
20.	<i>Lannea coromandelica</i> (Houtt.) Merr.	Anacardiaceae	Jhingan (H)	Wood; gum
21.	<i>Litchi chinensis</i> (Gaerth.) Sonner.	Sapindaceae	Litchi	Fruits edible
22.	<i>Litsea Stocksii</i> HK. f.	Lauraceae	Betel nut laurel	Timber
23.	<i>Macaranga peltata</i> (Roxb.) Muell.-Arg.	Euphorbiaceae	Chanda(Mar); Chandakanne (Kan); Uppila (Mal); Boddi; (Tel)	Wood for matches, paper pulp; gum; shade plant
24.	<i>Mallotus philippensis</i> (Lamk.) Muell.-Arg.	Euphorbiaceae	Kamala (H)	Fruits yield dye; timber
25.	<i>Mammea suriga</i> (Buch. ex Roxb.) Kostern.	Guttiferae	Nagkesar	Wood useful; flowers scented
26.	<i>Mesua ferrea</i> Linn.	Guttiferae	Nagkesar (H); Nagappu (Tam)	Timber; flowers for cosmetics
27.	<i>Muntingia calabura</i> Linn.	Elaeocarpaceae	Singapur cherry	Fruits edible; shade
28.	<i>Nephelium lappaceum</i> Linn.	Sapindaceae	Rambutan; Ramboostan	Fruits edible; wood useful
29.	<i>Persea americana</i> Mill.	Lauraceae	Avocado	Fruits
30.	<i>Pongamia pinnata</i> (Linn.) Pierre	Fabaceae	Indian beech; Karanja(H); Pangam (Tam) Ganuga (Tel)	Non-edible oil; timber; firewood; medical
31.	<i>Sapindus</i> Spp.	Sapindaceae	Scope-nut; Ritha (H). Pavamkottai (Tam)	Fruits used as a soap; wood useful

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
32.	<i>Sapum sebiferum</i> (Lour.) Roxb.	Euphorbiaceae	Chinese tallow; Vilaiati Shisham (H)	Fat from seeds used in soap and candle
33.	<i>Schefflera</i> spp.	Araliaceae		Wood useful
34.	<i>Scheuchera oleosa</i> (Lour.) Oken	Sapindaceae	Kusun (H); Kusumb (Mar)	Host for lac insect; seed yields oil
35.	<i>Sophora secundiflora</i> DC.	Fabaceae	Coral Bean	Wood useful
36.	<i>Syzygium</i> spp.	Myrtaceae	Jambul, etc.	Fruit edible; timber
37.	<i>Swietenia</i> spp.	Meliaceae	Mahagani (H)	Timber
38.	<i>Tamarix</i> spp.	Tamaricaceae	Jhau (H). etc.	Twigs used for making baskets
39.	<i>Terminalia tomentosa</i> Wt. and Arn.	Combretaceae	Ain (Mar); Asan, Sadri (H); Mathi (Kan)	Timber; nest for Tasar silkworm
40.	<i>Toona ciliata</i> M. J. Roem.	Meliaceae	Red cedar	Wood useful for many purposes
41.	<i>Vateria indica</i> Linn	Dipterocarpaceae	White-dammar; Safed damar (H)	Gum-resin; oil from seeds; wood useful
42.	<i>Veronia monodid</i> DC.	Asteraceae	—	Wood useful; firewood
Sub-tropical and temperate climates				
1.	<i>Acer caesium</i> Wall. Ex Brandis	Aceraceae	Trekhan (P); Kinar (Kashmir); Kilu (Kumaon)	Timber
2.	<i>Aesculus indica</i> Colebr. Ex Camb.	Hippocastanaceae	Horse chestnut; Kanor (H)	Nuts edible; timber
3.	<i>Alnus nepalensis</i> D. Don	Betulaceae	Indian alder; Utis (H); Kochi (P)	Timber
4.	<i>Alnus nitida</i> Endl.	Betulaceae	Kunis (H); Sharol (P); Chamb (Kashmir)	Timber
5.	<i>Castanea sativa</i> Mill	Fagaceae	Sweet chest-nut	Timber; Seed edible
6.	<i>Cordia dichotoma</i> Forst. F.	Ehretiaceae	Lasora (H); Vidi Tam	Timber; Fruit edible
7.	<i>Diospyros Kaki</i> L. f.	Ebenaceae	Persimmon	Fruits
8.	<i>Ehretia acuminata</i> R. Br.	Ehretiaceae	Ivory wood; Punia(H); Kulaaja (Beng)	Fruits edible; timber
9.	<i>Elaeocaroy</i> s spp.	Elaeocarpaceae	Rudrasa etc.	Timber
10.	<i>Eriobotrya japonica</i> (Thumb.) Lindl.	Rosaceae	Japanese Medlar; Loquat; (H)	Fruits
11.	<i>Eucalyptus</i> spp.	Myrtaceae	Neelgiri	Timber; paper pulp etc.
12.	<i>Gmelina arborea</i> Linn.	Verbenaceae	Ghamar(H); Kattanam(Tam)	Timber; fodder
13.	<i>Grewia</i> spp.	Tiliaceae	Phalsa (H)	Timber; fruits edible
14.	<i>Juglans regia</i> Linn.	Juglandaceae	Akhrot (H)	Fruits edible; wood useful

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
15.	<i>Legerstroemia</i> spp.	Lythraceae	Jarul; Harsingar (H)	Timber; ornamental
16.	<i>Litchi chinensis</i> (Gaertn.) Sonner	Sapindaceae	Litchi	Fruits
17.	<i>Litsea stocksii</i> HK. f.	Lauraceae	Betelnut laurel; Pisi (Mar); Varikeera (Mal)	Timber
18.	<i>Macaranga peltata</i> (Roxb.) Muell.-Arg.	Euphorbiaceae	Chanda (Mar); Boddli (Tel); Uppila (Mal); Chanda Kanne (Kan)	Gum; shade plant
19.	<i>Macilus macrantha</i> Nees	Lauraceae	Ladder wood; Kallamavu (Tam); Uravu (Mal)	Timber
20.	<i>Mallotus philippensis</i> (Lamk.) Muell.-Arg.	Euphorbiaceae	Kamala (H)	Timber; fruits yield dye
21.	<i>Moringa oleifera</i> Lamk.	Moringaceae	Drum-stick; Sainjana (H) Murangai (Tam); Shevaga (Mar); Munaga (Tel)	Leaves, flowers and fruits used as vegetables; medicinal
22.	<i>Muntingia calabura</i> Linn.	Elaeocarpaceae	Singapur-cherry	Fruits edible
23.	Orchard plants including citrus, apples, cherries, pears, plums, berries and nuts			Fruits and nuts
24.	<i>Paulownia tomentosa</i> Steud.	Scrophulariaceae	Himalayan Poplar	Wood useful
25.	<i>Populus ciliata</i> Wall. ex Royle	Salicaceae	Piplas	Timber
26.	<i>Quercus dilatata</i> Lindl.	Fagaceae	Moru	Timber; Fodder
27.	<i>Robinia pseudoacacia</i> Linn.	Fabaceae	False acacia	Timber
28.	<i>Salix</i> spp.	Salicaceae	Indian willow	Timber
29.	<i>Sapindus mukorossi</i> Gaertn.	Sapindaceae	Soapnut; Ritha (H); Reetha (P); Puvomkottai (Tam)	Timber; fruits as soap
30.	<i>Sapium sebiferum</i> (Linn.) Roxb.	Euphorbiaceae	Chinese Tallow-tree; Viliatti shisham (H)	Fat from seed used in soap and candles
31.	<i>Schleichera oleosa</i> (Lour.) Oken	Sapindaceae	Kusum (H); Kusumb (Mar)	Host for lac-insect; seed yields oil
32.	<i>Swietenia</i> spp.	Meliaceae	Mahagani (H)	Timber
33.	<i>Symplocos taurina</i> (Retz.) Wall. ex Rehder	Symplocaceae	Lodh	Wood as fuel
34.	<i>Syzgium cumini</i> Skeels	Hyrtaceae	Black plum; Jamun (H)	Fruits; medicinal
35.	<i>Toona ciliata</i> M.J. Roem	Meliaceae	Toon	Wood

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
Water courses/ canals				
1.	<i>Alseodaphne semcarifolia</i> Nees	Lauraceae	Phudgus (Mar); Yavarana (Tam); Massi (Kan)	Timber (grows in cooler climates)
2.	<i>Bischofia javanica</i> Bl.	Bischofiaceae	Bishop wood; Paniala (H); Boke (Mar); Nalupumushiti (Tel)	Wood useful for planking, buildings etc., railway sleepers
3.	<i>Calophyllum inophyllum</i> Linn.	Guttiferae	Alexandrian laurel; Sultana champ (H); Pin-nakottai (Tam)	Wood useful; seed yields oil
4.	<i>Dipterocarpus</i> spp.	Dipterocarpaceae	Black dammar	Valuable timber
5.	<i>Elaeis guineensis</i> Jacq.	Arecaceae	Red oil palm	Palm oil from fruits
6.	<i>Melaleuca leucodendron</i> Linn.	Myrtaceae	Kayapati (H); Kaiyappudai (Tam)	Leaves yield oil
7.	<i>Populus</i> spp.	Salicaceae	Indian willow; Bahan (H)	Wood for match boxes, packing cases, etc.
8.	<i>Salix</i> spp.	Salicaceae	Indian willow; Bis (H)	Wood for gun powder, charcoal, cricket bats
9.	<i>Syzygium cumini</i> Skeels	Myrtaceae	Black plum; Jamun (H)	Fruits edible; wood useful medicinal
10.	<i>Terminalia chebula</i> Retz.	Combretaceae	Gall-nut Myrabolani; Hard (H); Kadukai (Tam); Hirda Mar	Timber; bark for tanning and dyeing; fruits medicinal
11.	<i>Vitex leucoxylon</i> Linn.	Verbenaceae	Chirai gori (H)	Wood for furniture and carts
Sea coasts				
1.	<i>Anacardium occidentale</i> Linn.	Anacardiaceae	Cashewnut; Kaju (H)	Cashew apple and kernel edible, shell oil useful
2.	<i>Borassus flabellifer</i> Linn.	Arecaceae	Palmyra; Tal (H and Beng) Tad (Mar and Guj); Panei (Tam)	Used for posts, rafters etc.; Leaves for thatching mats, fans, basket work; fruits edible; Sap of peduncle for 'toddy' and palm sugar
3.	<i>Cocos nucifera</i> Linn.	Arecaceae	Coconut Palm; Nariyal (H); Thenmai (Tam); Tengu (Mal)	Oil, copra, fibre
4.	<i>Eucalyptus gomphocephala</i> A. Dc.	Myrtaceae	Turat	Timber; oil

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
5.	<i>Thespesia populnea</i> (Linn.) Soland ex Corr.	Malvaceae	Indian tulip; Paraspipal (H); Bhend (Mar); Poovarasu (Tam)	Bark yields fibre and dye; wood useful
Plants suggested for social forestry				
1.	<i>Aegle marmelos</i> (Linn.) Corr.			
		Rutaceae	Bael tree; Bel (H); Vilvam (Tam); Maredu (Tel)	Fruits edible; medicinal; timber
2.	<i>Aesculus indica</i> Colebr. ex Camb.	Hippocastanaceae	Horse chest-nut; Pangar (H)	Nuts edible; Timber
3.	<i>Antheopthalus cadamba</i> (Roxb.) Miq.	Rubiaceae	Kadamba (H); Kadam (Beng); Vellaicadamba (Tam)	Wood useful; flowers fragrant
4.	<i>Averrhoa carambola</i> Linn.	Averrhoaceae	Carambola tree	Fruit edible
5.	<i>Casmea sativa</i> Mill.	Fagaceae	Sweet chest-nut	Timber; seed edible
6.	<i>Ceiba pentandra</i> (Linn.) Gaertn.	Bombacaceae	White silk cotton; safedsimul(H); Ilavu(Tam); Katesawar(Mar)	Seed fibre; timber
7.	<i>Cinnamomum zeylanicum</i> Bl.	Lauraceae	Cinnamon, Dalchini(H); Ilayangam (Tam)	Bark yield spice
8.	<i>Cocos nucifera</i> Linn.	Arecaceae	Coconut tree; Nariyal (H); Thenai (Tam)	Oil from the nut, copra; Fibre from husk, etc.
9.	<i>Diospyros kaki</i> Linn. f.	Ebenaceae	Persimmon Fruits	Palm oil from fruits; ornamental
10.	<i>Elaeis guineensis</i> Jacq.	Arecaceae	African oil palm	Timber; seeds made into rosaries
11.	<i>Elaeocarpus</i> spp.	Elaeocarpaceae	Rudraksham	Fruits edible; medicinal
12.	<i>Embllica officinalis</i> Gaertn.	Euphorbiaceae	Embllic myrabolan, Amla (H); Avla (Mar); Lokat (H)	Fruits
13.	<i>Eriobotrya japonica</i> (Thumb.) Lindl.	Rosaceae		
14.	<i>Eucalyptus</i> spp.	Myrtaceae	Neelgiri	Timber; oil from leaves
15.	<i>Euphorbia longan</i> (Lour.) Steud.	Sapindaceae	Longan; Ashpal (Beng); Wumb (Mar); Puvatti(Tam)	Ornamental; shade; fruits
16.	<i>Garcinia indica</i> Chois	Guttiferae	Red mango Fruits edible	Timber; Fodder
17.	<i>Gmelina arborea</i> Linn.	Verbenaceae	Ghamar (H); Kattanam (Tam)	Fruits edible
18.	<i>Grewia</i> spp.	Tiliaceae	Phalsa, etc.	Wood used; shade tree; ornamental
19.	<i>Grevillea robusta</i> A. Cunn.	Proteaceae	Silver oak	Fruits edible; wood useful
20.	<i>Juglans regia</i> Linn.	Juglandaceae	Akhrot	Wood; ornamental
21.	<i>Lagerstroemia</i> spp.	Lythraceae	Jarul, Harsingar (H)	

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
22.	<i>Leucaena Leucocephala</i> (Lamk.) de Wit	Mimosaceae	Soobabul (H and Mar); Tagarai (Tam)	Green manure; fodder; firewood; timber
23.	<i>Limonia acidissima</i> Linn.	Rutaceae	Wood apple; Kait (H); Velaga (Tel); Kavath (Mar)	Fruits edible; gum; wood
24.	<i>Litchi chinensis</i> (Gaertn.) Sonner.	Sapindaceae	Litchi Fruits edible	Medicinal
25.	<i>Moringa oleifera</i> Lamk.	Moringaceae	Drum stick tree; Sainjana (H); Murangai (Tam); Shevga (Mar); Munaga (Tel)	Fruits edible; shade
26.	<i>Muntingia calabura</i> Linn.	Elaeocarpaceae	Singapur-cherry	Fruits; wood useful
27.	<i>Nephelium lappaceum</i> Linn.	Sapindaceae	Rambutani; Ramboostan berries and nuts	Fruits and nuts
28.	Orchard plants-citrus, apples, cherries, pears, plums,			
29.	<i>Persea americana</i> Mill.	Lauraceae	Avocado	Fruits
30.	<i>Pongamia pinnata</i> (Linn.) Pierre	Fabaceae	Indian beech; Karanja (H and Mar); Pangam(Tam); Ganuga (Tel)	Non-edible oil; medicinal; timber; firewood
31.	<i>Sapindus</i> spp.	Sapindaceae	Soapnut, Ritha (H); Puvamkottai (Tam)	Fruits used as soap; timber
32.	<i>Sapium sebiferum</i> (Linn.) Roxb.	Euphorbiaceae	Vilaiti shisham (H)	Fat from seeds used in soap and candle
33.	<i>Sesbania grandiflora</i> Pers.	Fabaceae	Basna (H); Avesi (Tel); Hadega (Mar)	Vegetable; firewood
34.	<i>Syzgium cumini</i> Skeels	Myrtaceae	Black plum; Jamun(H); Naval(Tam); Jam-bul(Mar)	Fruits edible; medicinal
35.	<i>Tamarindus indica</i> Linn.	Caesalpiniaceae	Tamrind tree; Imli (H); Tentul (O) Puli (Tam and Mal)	Fruits and young leaves edible; condiment; firewood, timber
36.	<i>Ziziphus mauritiana</i> Lamk.	Rhamnaceae	Indian cherry plum;Ber (H); Hanthai (Tam)	Fruits edible; fodder; firewood
Plants suggested for crop rotation in agricultural farms				
1.	<i>Amaranthus caudatus</i> Linn.	Amaranthaceae	Ram-dana(H); nantmul(Beng)	Food grain
2.	Brassica spp.	Brassicaceae	Mustards and Rapeseed	Oil-seed crop
3.	<i>Coriandrum sativum</i> Linn.	Apiaceae	Coriander; Dhaniya (H); Kothamalli (Tam)	Condiment; medicinal
4.	<i>Cucumis sativus</i> Linn.	Cucurbitaceae	Cucumber; Kheera (H); Kakdi (Mar)	Vegetable
5.	<i>Fagopyrum esculentum</i> Moench	Polygonaceae	Buckwheat; Kutu (H)	Food grain crop; medicinal

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
6.	<i>Guzotia abyssinica</i> Cass.	Asteraceae	Niger; Kalatil (H); Valesulu (Tel)	Oil seed crop
7.	<i>Helianthus annuus</i> Linn.	Asteraceae	Sunflower; Suryamukhi (H); Suryaphul (Mar)	Oil seed crop
8.	<i>Medicago sativa</i> Linn.	Fabaceae	Lucerne; Lusan (P); Lasun ghas (Mar)	Fodder
9.	<i>Mellilotus indica</i> (Linn.) All.	Fabaceae	Sweet clover; Senji (H)	Fodder
10.	<i>Phaseolus lunatus</i> Linn.	Fabaceae	Double bean; Ban barbat (Beng)	Vegetable
11.	<i>Raphanus sativus</i> Linn.	Brassicaceae	Radish; Mooli (H); Mula (Mar)	Vegetable
12.	<i>Sorghum bicolor</i> (Linn.) Moench	Poaceae	Sorghum, Jowar	Fodder grain; fodder
13.	<i>Trifolium alexandrinum</i> Linn.	Fabaceae	Egyptian clover; Berseem (H and P)	Fodder
14.	<i>Zea mays</i> Linn.	Poaceae	Maize, Makka (H); Mokka jonna (Tel)	Food grain; fodder
Hedge plants				
1.	<i>Acnistatus arborescens</i> Schlect.	Solanaceae	–	Ornamental; fruit for birds
2.	<i>Adhatoda vasica</i> Nees	Acanthaceae	Bakas (H and Ben); Adathodai (Tam)	Green manure; amenity; medicinal
3.	<i>Buddleia madagascariensis</i> Lamk.	Loganiaceae	Buddleia	Ornamental
4.	<i>Duranta repens</i> Linn.	Verbenaceae	Golden dew drop	Ornamental
5.	<i>Euphorbia antiquorum</i> Linn.	Euphorbiaceae	–	Ornamental
6.	<i>Jatropha gossypifolia</i> Linn.	Euphorbiaceae	Bellyache Bush	Ornamental
7.	<i>Justicia gendarussa</i> Linn. f.	Acanthaceae	Willow leaved justicia	Ornamental
8.	<i>Lagerstroemia indica</i> Linn.	Lythraceae	Pride of India	Ornamental
9.	<i>Lawsonia inermis</i> Linn.	Lythraceae	Indian Privet; Mehandi (H); Goranti (Kan)	Leaves yield dye
10.	<i>Synadenium grantii</i> Hk. f.	Euphorbiaceae	African milk-bush	Ornamental
11.	<i>Vitex negundo</i> Linn.	Verbenaceae	Nigandi (H); Nirgundi (Beng); Vennochi (Tam and Mal)	Reclamation of forest land; medicinal
Avenue trees				
1.	<i>Acacia auriculiformis</i> A.Cun. ex Benth.	Mimosaceae	Australian Phyllode acacia	Firewood
2.	<i>Ailanthus excelsa</i> Roxb.	Simaroubaceae	Mahanuk (H and Mar); Perumaram (Tam)	Wood useful
3.	<i>Albizia</i> spp.	Mimosaceae	Siris (H); etc.	Wood useful; shade tree

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
4.	<i>Azadirachta indica</i> A. Juss.	Meliaceae	Neem (H); Vaepparamaram (Tam)	Timber
5.	<i>Dalbergia</i> spp.	Fabaceae	Shisham(H); etc.	Timber (durable)
6.	<i>Delonix regia</i> (Boj) Rafin.	Caesalpinaceae	Gul-mohar (H)	Shade, ornamental
7.	<i>Dillenia indica</i> Linn.	Dilleniaceae	Chalta (H and Beng); Karamal (Mar and Guj) Uvu (O)	Timber; jam and jellies from fruits
8.	<i>Ehretia acuminata</i> R. Br.	Ehretiaceae	Ivory wood	Fruits edible
9.	<i>Elaeocarpus</i> spp.	Elaeocarpaceae	Rudraksham	Timber
10.	<i>Eucalyptus</i> spp.	Myrtaceae	Neelgiri	Timber
11.	<i>Filicium dicipiens</i> Thw.	Sapindaceae	Ningal (Tam); Niroli (Mal)	Timber
12.	<i>Grevillea robusta</i> A. Cunn.	Proteaceae	Silver oak	Timber; shade tree
13.	<i>Melia azedarach</i> Linn.	Meliaceae	Bakain(H); Malaivembu(Tam)	Wood; medicinal
14.	<i>Parkinsonia aculeata</i> Linn.	Caesalpinaceae	Jarusalam Thorn; Vilayati Kilkar (H)	Ornamental
15.	<i>Peltophorum pterocarpum</i> (DC.) Backer ex K. Heyne	Caesalpinaceae	Copper pod; Konda Chinta (Tel)	Ornamental; wood useful
16.	<i>Pongamia pinnata</i> (Linn.) Pierre	Fabaceae	Indian beech; Karanja (H); Pangam (Tam); Ganuga (Tel)	Non-edible oil; medicinal, timber, firewood
17.	<i>Populus</i> spp.	Salicaceae	Himalayan Poplar; Piplas	Timber
18.	<i>Robinia pseudoacacia</i> Linn.	Fabaceae	False acacia	Timber
19.	<i>Samanea saman</i> (Jacq.) Merr.	Mimosaceae	Rain tree; Vilati siris (H)	Firewood
20.	<i>Spathodea campanulata</i> Beauv.	Bignoniaceae	Scarlet bell	Ornamental
21.	<i>Swietenia mahagoni</i> (Linn.) Jacq.	Meliaceae	Mahagani (H)	Timber
22.	<i>Syzygium cumini</i> Skeels	Myrtaceae	Black Plum; Jamun (H); Jambul (Mar)	Fruit edible
23.	<i>Tabebuia</i> spp.	Bignoniaceae	Basant rani(H); etc.	Ornamental
24.	<i>Tamarindus indica</i> Linn.	Caesalpinaceae	Tamarind tree; Imli (H); Tentul (O); Puli (Tam)	Timber; condiment; firewood; fruits and young leaves edible
25.	<i>Thespesia populnea</i> (Linn.) Soland. ex Correa	Malvaceae	Indian tulip; Paras-pipal (H); Poovarasu (Tam)	Bark fibre

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
Ornamental trees				
1.	<i>Acacia auriculiformis</i> A.Cunn. ex Benth.	Mimosaceae	Australian Phyllode acacia	Firewood
2.	<i>Albizia procera</i> Benth.	Mimosaceae	White siris tree; Velvagai (Tam)	Timber, medicinal
3.	<i>Citharexylum subserratum</i> Sw.	Verbenaceae	Fiddlewood tree	Ornamental
4.	<i>Erythrina crista-galli</i> Linn.	Fabaceae	Dadap, etc.	Wood useful
5.	<i>Eucalyptus ficifolia</i> F. Muell.	Myrtaceae	Red-flowering gum	Wood
6.	<i>Guaiacum officinale</i> Linn.	Zygophyllaceae	Lignum Vitae tree	Wood useful; resin
7.	<i>Kleinhovia hospita</i> Linn.	Sterculiaceae	Kleinhovia; Panaitteku (Tam)	Medicinal; wood useful
8.	<i>Kydia calycina</i> Roxb.	Myrtaceae	Roxburghs Kydia; Pola (H); Potari (Tel)	Bark fibre; wood useful
9.	<i>Lagerstroemia speciosa</i> (Linn.) Pers.	Lythraceae	Jarul (H)	Wood useful
10.	<i>Muntingia calabura</i> Linn.	Elaeocarpaceae	Singapur-cherry	Fruits edible
11.	<i>Parkinsonia aculeata</i> Linn.	Casalpiniaceae	Jerusalem thorn; Vilayati Kikar (H)	Fuel wood; fodder
12.	<i>Paulownia tomentosa</i> Steud.	Scrophulariaceae	–	Excellent wood
13.	<i>Sophora viciifolia</i> Hance	Fabaceae	–	Timer (grown in cooler climates)
14.	<i>Spathodea campanulata</i> Beauv.	Bignoniaceae	Scarlet-bell	Ornamental
15.	<i>Syzygium</i> spp.	Myrtaceae	–	Timber
16.	<i>Tabebuia</i> spp.	Bignoniaceae	Basant rani (H); etc	–
Shrubs				
1.	<i>Acacia farnesiana</i> Willd.	Mimosaceae	Fragrant acacia	Bark yield tannin
2.	<i>Calliandra tweedi</i> Benth.	Mimosaceae	Calliandra	–
3.	<i>Callistemon linearis</i> DC.	Myrtaceae	Bottle brush	Bark from fibre
4.	<i>Dombeya mastersii</i> HK. f.	Sterculiaceae	Dombeya	–
5.	<i>Hamelia patens</i> Jack.	Rubiaceae	The spreading hamelia	–
6.	<i>Murraya paniculata</i> (Linn.) Jack.	Rutaceae	Orange Jasmine	–
7.	<i>Rosa moschata</i> Mill.	Rosaceae	Wild rose	–

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
8.	<i>Russelia equisetiformis</i> Schlecht. and Cham.	Scrophulariaceae	Bright coral-red	—
9.	<i>Serrissa foetida</i> Lamk.	Rubiaceae	—	—
10.	<i>Tamarix</i> spp.	Tamaricaceae	Tamarisk	Twigs used for making baskets
Climbers				
1.	<i>Antigonon leptopus</i> HK. and Arn.	Polygonaceae	Antigonum	—
2.	<i>Jacquemonita pentantha</i> (Jacq.) G. Don	Convolvulaceae	—	—
3.	<i>Pyrostegia venusta</i> (Ker-Gawl.) Miers	Bignoniaceae	Golden shower	—
4.	<i>Porana volubilis</i> Burm.	Convolvulaceae	—	—
5.	<i>Quisqualis indica</i> Linn.	Combretaceae	Rangoon creeper	—
6.	<i>Wisteria sinensis</i> DC.	Fabaceae	Grape flower wine	—
Herbs				
1.	<i>Ageratum conyzoides</i> Linn.	Asteraceae	Ageratum	—
2.	<i>Althaea rosea</i> (Linn.) Cav.	Malvaceae	Hollyhock	—
3.	<i>Bellis perennis</i> Linn.	Asteraceae	Daisy	—
4.	<i>Calendula officinalis</i> Linn.	Asteraceae	Calendula	—
5.	<i>Callistephus chinensis</i> (Linn.) Nees	Asteraceae	Aster	—
6.	<i>Celosia argentea</i> Linn.	Amaranthaceae	Cockscomb	—
7.	<i>Centaurea cyanus</i> Linn.	Asteraceae	Corn flower	—
8.	<i>Coleus amboinicus</i> Lour.	Lamiaceae	Coleus	—
9.	<i>Coreopsis drummondii</i> Torr. and Gray	Asteraceae	Coreopsis	—
10.	<i>Cosmos bipinnatus</i> Cav.	Asteraceae	Cosmos	—
11.	<i>Cosmos sulphureus</i> Cav.	Asteraceae	Cosmos	—

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
12.	<i>Dahlia</i> spp.	Asteraceae	Dahlia (Singles)	
13.	<i>Gomphrena globosa</i> Linn.	Amaranthaceae	Globe Amaranth	
14.	<i>Gypsophila cerastioides</i> D. Don	Caryophyllaceae	Gypsophila	
15.	<i>Ocimum sanctum</i> Linn.	Lamiaceae	Tulsi (H); Tulasi (Kan and Guj)	
16.	<i>Papaver rhoeas</i> Linn.	Papaveraceae	Lal-post (H)	
17.	<i>Phlox drummondii</i> HK.	Polemoniaceae	Drummond Phlox	
18.	<i>Pimpinella monoica</i> Dalz.	Apiaceae	Lady's Lace	
19.	<i>Polianthus tuberosa</i> Linn.	Agavaceae	Tuberose	
20.	<i>Portulaca grandiflora</i> HK.	Portulacaceae	Portulaca	
21.	<i>Salvia</i> spp.	Lamiaceae	Sage	
22.	<i>Solidago canadensis</i> Linn.	Asteraceae	Golden rod	
23.	<i>Tagetes erecta</i> Linn.	Asteraceae	Marigold; Genda (H)	
24.	<i>Tradescantia virginiana</i> Linn.	Commelinaceae	–	
25.	<i>Zinnia</i> spp.	Asteraceae	Zinnia	
Non-littoral high salinity and water scarcity conditions				
Sr.	Botanical name	Family	Economic use	Ecological preference
1.	<i>Eucalyptus magacornuta</i> C.A.	Myrtaceae	Shade tree, leaves rich in oil	–
	Garden		sheler belts	Saline soils, shifting sands
2.	<i>Tamarix articulata</i> (aphylla)	Tamaraceae	timber useful, fire wood	
3.	<i>T. diosca</i> Roxb.	Tamaraceae	Branches used for making baskets	River beds and sea coasts
4.	<i>Thespesia populnea</i> (Linn.) Soland, ex Correa	Malvaceae	Shade	–
Along backwaters				
	Botanical Name	Family	Occurrence	Economic use
	<i>Sapium indicum</i> Willd.	Euphorbiaceae		Seed yield oil, fruits for fish poison, root bark medicinal

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
	<i>Hibiscus tiliaceus</i> Linn.	Malvaceae		Bark fibre used for many purposes, leaves for cattle feed; root medicinal
	Fresh Water Swamps			
	<i>Myristica magnifica</i> Bedd.	Myristicaceae		
	<i>M. laurifolia</i>	Myristicaceae		
	<i>M. canarica</i>	Myristicaceae		
	<i>M. malabarica</i> Lam.	Myristicaceae		
	<i>Lagerstroemia speciosa</i> Pers	Lythraceae		Seed oil used for illumination
	<i>Lophopetalum wightianum</i> Arn.	Celastraceae		Wood for structural work flooring and interior fittings
	<i>Eugenia montana</i>	Myrtaceae		Timber
	<i>Carallia brachiata</i>	Rhizophoraceae		Fruits edible, and also used in treatment of contagious ulcers
	<i>Eucalyptus ptychocarpa</i> F. Muell.	Myrtaceae		Very attractive, wood useful
	Mangrove bee plants (In the order of their distance from sea water)			
	Botanical Name	Family	Economic use	Ecological preference
	<i>Avicennia officinalis</i> Linn.	Avicenniaceae	Wood for fuel, leaves used as cattle fodder	Tidal forests, Near high tide level salt marshes
	<i>Bruguiera gymnorrhiza</i> (Linn.) Lamk.	Rhizophoraceae	Fire-wood, wood used in house construction	Swampy regions normal high tide level
	<i>Kandelia candel</i> (Linn.) Druce	Rhizophoraceae	Green manure, wood for charcoal, fuel	Swampy regions
	<i>Rhizophora mucronata</i> Lamk.	Rhizophoraceae	Timber, bark for tannin, fruits edible	Bed level, swampy regions normal high tide level
	<i>Sonneratia acida</i> Benth.	Sonneratiaceae	Fruits eaten, medicinal, wood useful	Swampy regions
	<i>Cressa cretica</i> Linn.	Convolvulaceae	–	Saline marshes and coastal sands
	<i>Acanthus ilicifolius</i> Linn.	Acanthaceae	Cattle fodder	Further towards land
	<i>Clorodendrum inerme</i> (Linn.) Gaertn.	Verbenaceae	Ornamental	Further towards land
	<i>Aegiceras corniculatum</i> (Linn.) Blanco	Myrsinaceae	Bark for tanning, wood for fuel	Spring high tide level
	<i>Amoora cucullata</i> Roxb.	Maliaceae	Wood for posts and fire wood	On the landward higher land

Table 12.8 (continued)

Sr. No.	Botanical name	Family	Common/local names	Economic use
	Barringtonia racemosa (Linn.)	Spring	Barringtoniaceae	Higher land
	Calophyllum inophyllum Linn.	Guttiferae	Fire wood, fruit, seed and root medicinal	Higher land
	Dalbergia spinosa Roxb.	Fabaceae	Timber, seed-oil used in skin diseases etc	Higher land
	Excoecaria agallocha Linn.	Euphorbiaceae	Wood for match industry	Spring high tide level
	Heritiera littoralis Dryand	Sterculiaceae	Timber, seeds edible, bark for tannin	Higher land
	Hibiscus tiliaceus Linn.	Malvaceae	Bark fibre, leaves for cattle feed, root medicinal	Near back waters, tidal streams, along river banks
	Salvadora persica Linn.	Salvadoraceae	Wood useful	Saline soil
	Xylocarpus granatum Koen	Meliaceae	Wood valuable	Higher land
	X. molluccensis (Lamk.) Roem.	Meliaceae	Carapa-oil from seeds	Higher land
	Areca triandra Roxb.	Areaceae	—	—
	Nypa fruticans Wurm.	Areaceae	Sweet sap for jaggery, sugar, alcohol and vinegar. Leaves useful	Mangrove swamps, tidal forests
	Phoenix pludoss Roxb.	Areaceae	Leaves useful; fruits and pith of the stem edible	Spring tides on drier areas within salt water mangroves

when there is no honey flow it is called a dearth period. Some examples of suitable localities, based on the availability of bee pasturages in India are described below, as beekeeping is only profitable if bee pasturage is abundantly available in a particular locality.

More the number of blossom periods in a year in an area, greater will be the potential for beekeeping. Even if the number of blossoming periods are few but the duration is longer that favours beekeeping. Whether bees are visiting for pollen or nectar can be noted by observing the bees on the flowers or on the entrance of hive. This observation can provide us the type of flowering plants (nectar bearing or pollen bearing or both) in an area at a particular period of the year and also whether the bees are more in need of pollen or nectar or both. Area with special crops having more nectar or pollen has a higher suitability for beekeeping. Similarly areas with more varieties of nectar secreting natural flower has a high favourable condition for beekeeping. Shorter the dearth period greater will be the bee pasturage. Areas close to (within 2–6 km) nectar and pollen bearing plant and crops are suitable for beekeeping. Finally the favourable weather conditions such as temperature between 15–35 °C, wind speed less than 30 km/h, clear sunny days are favourable for bee pasturage. Too cold, hot and rainy days prevent bee from pasturage.

12.12 Foraging Bees for Pollen Collection

In recent years, there has been an ever increasing interest in employing honeybees to collect pollen for various uses in human diet, plant breeding, etc. Demand for pollen has increased markedly and many beekeepers are now involved in pollen collection and sale, even though it results in decreased honey production. Pollen is collected through bees by attaching to the beehive, a special device called pollen trap.

12.13 Case Studies

12.13.1 *Honeybee Flora in Thailand*

Bee flora, or bee plants, is the plants from which bees collect pollen and nectar. Honeybees forage on a variety of plant species to collect nectar and pollen (McGregor 1976), including agricultural crops and native plants. They are particularly efficient pollinators for native plants due to the morphological structure of their organs and external features, such as hairs that cover their body to help carry nectar and pollen (Suwannapong et al. 2012). Different honeybees that have different morphology may affect their foraging preferences (Table 12.9).

However, not all plant species are bee flora. Many plant species, including agricultural crops and native plants, are pollinated by honeybees which are good pollinators particularly for native plants. They are such good native plant flora pollinators because they have morphological structures that facilitate pollen attachment, transfer

Table 12.9 Nectar, pollen and Nectar and pollen source plants of Thai honeybees. (Suwannapong et al. 2012)

Number	Plant species	Nectar source	Pollen source
1	<i>Ageratum conyzoides</i> L.	+	+
2	<i>Amomum xanthioides</i> Wall.	+	+
3	<i>Balakara baccata</i> Roxb.	+	+
4	<i>Blumea balsamifera</i> (L.) DC.	+	+
5	<i>Bidens biternata</i> Merr. and Sherff.	+	+
6	<i>Brachiaria ruziziensis</i> Germain and Evrard	—	+
7	<i>Brassica chinensis</i> Jusl var.	+	+
8	<i>Castanopsis acuminatissima</i> Rehd.	—	+
9	<i>Ceiba pentandra</i> (L.)	+	+
10	<i>Cinnamomum kerrii</i> Kosten	+	+
11	<i>Citrus aurantifolia</i> Swing.	+	+
12	<i>Citrus maxima</i> (J. Burman) Merr.	+	+
13	<i>Coccinia grandis</i> CL.Voigt	+	+
14	<i>Cocos nucifera</i> L.	+	+
15	<i>Coffea Arabica</i> L.		+
16	<i>Coriandrum sativum</i> L.	+	+
17	<i>Conyza sumatrensis</i> Retz.	+	+
18	<i>Crataeva magna</i> Lour.	+	+
19	<i>Croton oblongifolius</i> Roxb.	+	+
20	<i>Cuphea hyssopifolia</i> H.B.K.	+	—
21	<i>Dalbergia oliveri</i> Gamble ex Prain	+	
22	<i>Datura metel</i> L.	+	+
23	<i>Dillenia ovata</i> Wall.	+	+
24	<i>Dimocarpus longan</i> Lour.	+	+
25	<i>Diospyros glandulosa</i> Lacc.	+	+
26	<i>Diospyros areolata</i> King and Gamble	+	+
27	<i>Duabanga grandiflora</i> Walp.	+	+
28	<i>Elaeagnus latifolia</i> L.	+	+
29	<i>Erythrina suvumbrans</i> Merr.	+	+
30	<i>Eucalyptus camaldulensis</i>	+	+
31	<i>Eugenia javanica</i>	+	+
32	<i>Eupatorium odoratum</i> L.	+	+
33	<i>Euphoria longana</i> Lamk.	+	+
34	<i>Fragaria ananassa</i> Guedes	+	+
35	<i>Gmelina arborea</i> Roxb.	+	+
36	<i>Hopea odorata</i> Roxb.	+	+
37	<i>Jacaranda filicifolia</i> D.Don	+	+
38	<i>Leersia hexandra</i> Sw. - +	—	+
39	<i>Leucaena leucocephala</i> Wit.	—	+
40	<i>Litchi chinensis</i> Sonn	+	+
41	<i>Macadamia integrifolia</i> maiden and Bette	+	+
42	<i>Mangifera indica</i> L.	+	+
43	<i>Mikania cordata</i> Roxb.	+	+

Table 12.9 (continued)

Number	Plant species	Nectar source	Pollen source
44	<i>Mimosa diplotricha</i> C. Wright.	+	+
45	<i>M. pigra</i>	+	+
46	<i>M. pudica</i> L.	—	+
47	<i>Muntingia calabura</i> L.	+	+
48	<i>Musa acuminata</i> Colla.	+	+
49	<i>M. sapientum</i> L.	+	+
50	<i>Ocimum sanctum</i> L.	+	+
51	<i>Oryza sativa</i> L.	—	—
52	<i>Oxalis acetosella</i> L.		+
53	<i>Passiflora laurifolia</i> L.	+	+
54	<i>Prunus cerasoides</i> D.Don	+	+
55	<i>P. mume</i> Sieb.	+	+
56	<i>Psidium guajava</i> L.	+	+
57	<i>Raphanus sativus</i> L.	+	+
58	<i>Schoenoplectus juncooides</i> (Roxb.) Palla.	—	+
59	<i>Shorea siamensis</i> Miq.	+	—
60	<i>Solanum torvum</i> SW.	+	+
61	<i>Spilanthes paniculata</i> Wall. Ex DC.	+	+
62	<i>Synedrella nodiflora</i> (L.) Gaerth.	+	+
63	<i>Wedelia trilobata</i> (L.) Hiteh.	—	+
64	<i>Wrightia arborea</i> (Dennst.) Mabb.	+	+
65	<i>Zea mays</i> L.	—	+
66	<i>Zizyphus mauritiana</i> Lamk.	+	—

and deposition. For instances, they have a proboscis with the appropriate length and shape to match specific morphology of certain flowers. They also have a body covered with hairs and setae that adhere pollen and pollen baskets that are adaptations for carrying pollen by carrying a static electrical charge. This helps pollen (and other small particles) stick to them (Suwannapong et al. 2012). However, a plant that produces nectar and pollen prolifically in one geographic region may not yield the same amount of nectar and pollen in another region (Erdtman 1966, 1969; Latif et al. 1960; Singh 1981). There are three types of bee flora: plants that only supply nectar, plants that only supply pollen, and plants that provide both (Allen et al. 1998; Baker 1971; Baker and Baker 1983; Bhattacharya 2004; Crane et al. 1989; Partap 1997).

Some plants provide only resin, but these are less common. Floral nectar provides energy for flight activity, foraging activity and other activity in the colony. Honeybees also convert the nectar into honey and store it in the honey storage area of the comb. Pollen provides protein, lipids, minerals and vitamins (Gary 1975; 1992). Pollen from different plant species differs in nutritive value and attractiveness to honeybees (Baker 1971; Baker and Baker 1983; Erdtman 1966, 1969; Shuel, 1992; Suwannapong et al. 2012).

There are more than 30 species of plants visited by *A. andreniformis* in Thailand such as *Anacardium occidentale* L., *Antigonon leptopus* Hook., *Balakara baccata* Roxb., *Brassica chinensis* Jusl var., *Castanopsis acuminatissima* Rehd., *Chrysal*, *Cocos nucifera* L., *Coriandrum sativum* L., *Conyza sumatrensis* Retz., *Cucurbita*

citrillus L. *Cucumis sativus* Linn, *Cuphea hyssopifolia* H.B.K., *Dimocarpus longan* Lour., *Eugenia javanica* and *Mimosa pigra* (Suwannapong et al. 2012).

The plants visited by *A. florea* include more than 40 species such as *M. pigra*, *Callistemon viminalis*, *Vetchia merrillii* (Becc.) H.E. Mosre, *Cocos nucifera* L., *Melampodium divaricatum*, *Zea mays* L., *C. hyssopifolia* H.B.K., *D. longan* Lour., *Durio zibethinus* L., *E. javanica*, *Eupatorium odoratum* L., *Euphoria longana* Lamk., *Fragaria ananassa* Guedes, *Hopea odorata* Roxb (Maksong 2008; Suwannapong et al. 2012). Therefore, *A. dorsata* reportedly uses fewer food plants than *A. florea*. Only 38 species are reportedly used by *A. dorsata*: *Ageratum conyzoides* L., *Amomum xanthioides* Wall., *Anacardium occidentale* L., *Blumea balsamifera* L. DC., *Bidens biternata* Merr. and Sherff., *Celosia argentea*, *Cinnamomum kerrii* Kosten, *Citrus aurantifolia* Swing., *C. maxima* (J. Burman) Merr., *Cocos nucifera* L. (Maksong 2008; Suwannapong et al. 2012). However, few hundred plant species visited by *A. cerana*. There are more than 68 species of *A. cerana* bee plant in Thailand. These include *Aeschynomene americana* L., *Ageratum conyzoides* L., *Amomum xanthioides* Wall., *Anacardium occidentale* L., *Antigonon leptopus* Hook. Balakara baccata Roxb., *Bidens biternata* Merr. and Sher, *Brachiaria ruzizien-sis* Germain and Evrard, *Castanopsis acuminatissima* Rehd., *Cinnamomum kerrii* Kosten, *Coccinia grandis* CL.Voigt, *Cocos nucifera* L., *Coffea Arabica* L., *Conyza sumatrensis* Retz. The number of bee flora of the introduced honeybee species in Thailand are more than 54 species such as *Ageratum conyzoides* L., *Durio zibethinus* L., *Euphoria longana* Lamk., *Fragaria ananassa* Guedes, *Leersia hexandra* Sw., *Macadamia integrifolia* maiden and Betche, *Mikania cordata* Roxb., *Mimosa pigra*, *Musa acuminata* Colla., *Nephelium lappaccum* L., *Ocimum basilicum* L., *Oryza sativa* L., *Oxalis acetosella* L., *Prunus mume* Sieb., *Psidium guajava* L., *Sesamum indicum* L., *Schoenoplectus juncooides* (Roxb.) Palla, *Raphanus sativus* L. (Maksong 2008; Suwannapong et al. 2011). However, more than 100 crops in the United States are bee plants for *A. mellifera* such as *Abelmoschus esculentus*, *Actinidia deliciosa*, *Allium cepa*, *Anacardium occidentale*, *Apium graveolens*, *Arbutus unedo*, *Averrhoa carambola*, *Brassica alba*, *B. hirta*, *B. nigra*, *B. napus*, *B. oleracea* cultivar, *B. rapa*, *Cajanus cajan*, *Carica papaya*, *Carthamus tinctorius*, *Carum carvi*, *Castanea sativa*, *Citrullus lanatus*, *Citrus reticulata*, *Cocos nucifera*, *Coffea* spp., *Coriandrum sativum*, *Coronilla varia* L., *Cucumis melo* L., *Cucumis sativus*, *Cyamopsis tetragonoloba*, *Cydonia oblonga* Mill, *Daucus carota*, *Dolichos* spp. *Dimocarpus longan*, *Diospyros kaki*, *D. virginiana*, *Elettaria cardamomum*, *Eriobotrya japonica*, *Fagopyrum esculentum*, *Feijoa sellowiana*, *Foeniculum vulgare*, *Fragaria* spp., *Glycine max*, *G. soja*, *Helianthus annuus*, *Juglans* spp., *Linum usitatissimum*, *Lichi chinesis*, *Lupinus angustifolius* L., *Macadania ternifolia*, *Malpighia glabra*, *Malus domestica*, *Mangifera indica*, *medicago sativa*, *Nephelium lappaceum*, *Onobrychis* spp., *Persea Americana*, *Phaseolus* spp., *P. coccineus* L., *Pimenta dioica*, *Prunus armeniaca*, *P. avium* spp., *P. cerasus*, *P. domestica*, *P. spinosa*, *P. dulcis*, *P. amygdalus*, *P. persica*, *Psidium guajava*, *Punica granatum*, *Pyrus communis*, *Ribes nigrum*, *R. rubrum*, *Rosa* spp., *R. idaeus*, *R. fruticosus*, *Sambucus nigra*, *Sesamum indicum*, *Solanum melongena*, *Spondias* spp., *Tamarindus indica*, *Trifolium alba*, *T. hybridum* L., *T. incarnatum*, *T. pretense*, *T. vesiculosum*, *Vaccinium* spp.,

V. oxycoccus, *V. macrocarpon*, *Vercia faba*, *Vigna unguiculata*, *Vitellaria paradoxa* (http://en.wikipedia.org/wiki/list_list_of_crop_plants_pollinated_by_bees).

12.14 Melsisspalyanological Studies

Plants are essential to the honeybees' life. The production of honey depends on an abundant supply of nectar and pollen producing plants within easy flight range of the bee colony. Nectar forms the basis of honey, the energy rich (carbohydrate) food that honeybees need to sustain the life of the colony while pollen provides the protein, vitamins and other nutrients needed for the developing larvae. Without sufficient carbohydrate, the colony will die of starvation quite quickly; without pollen the colony will die out slowly as it will not be able to produce new bees to replace old ones as they die. Propolis is collected from the resinous exudations of a range of trees. This is used to strengthen the comb, to keep the hive clean and free of infection and to seal up holes keeping out leaks and draughts as well as robber bees and damaging intruders. Bees and flowering plants have a long relationship, developing together over the millennia in a way that benefited both the plants and the insects and enabling both to survive better than they would have done alone. Bees are important to humans, not only for the production of honey and wax but particularly for their role in the pollination of plants including some important economic crops.

Worldwide, about 40,000 plant species are of value to bees while about 4,000 are the source of most of the world's honey. Among a long list of important economic crops, bees have been shown to increase the yields of sunflower, oil-seeds, peaches, almonds, kiwi fruit, coffee, avocado, mango, pumpkins and beans. Beekeepers who have acquired a good knowledge of the floral sources in their locality will be able to maintain their colonies in the best places for getting honey. Part of the skill of beekeeping is to enable the maximum population of foraging bees at the time when there is the maximum number of flowers. This will give the bees the best chance of collecting as much nectar as they can—and of course for the beekeeper to utilise the resulting honey crop.

Pollen analysis of honeys encompassing qualitative and quantitative methodology is of great significance in apiculture. Identification of bee plants from diverse geographic areas and floristic communities helps immensely in the development of beekeeping industry. Melittopalynological studies have received to date only marginal attention in various parts of India. Nair (1964), Seethalakshmi (1980) and Chanda and Ganguly (1981) carried out pollen analysis of a few honey samples from Andhra Pradesh. During the last decade, serious efforts have been made in pollen analytical studies of honeys from this state. Jhansi and Ramanujam (1986, 1987, 1990) provided pollen analysis of some extracted and squeezed honeys from various parts of Andhra Pradesh. Kalpana and Ramanujam (1989, 1991) and Kalpana et al. (1990) and Jhansi et al. (1994) carried out detailed qualitative and quantitative pollen analytical studies of squeezed honeys of *A. florea* Fabr and *A. cerana* Fabr from the Ranga Reddy and Hyderabad districts.

12.14.1 *Bee Forage as Identified from Pollen Sources in Honey Samples*

Bees and flowering plants are mutually dependent as bees need flowering plants for food in the form of pollen and nectar, whereas plants need bees for pollination. Honey contains pollen grains which are collected by honeybees while foraging the flowers for pollen and nectar. The microscopic analysis of pollen is the standard method and an effective tool to understand the distribution and abundance of floral nectar sources in any given region. Studying the pollen in honey greatly contributes to the understanding of the geographical and botanical origin of honey, as the bees are known to visit more than 3 km in the search of forage.

Melissopalynology, one of the branches of palynology, finds a very significant application in the field of apiculture for recognizing the nectar sources and botanical origin of honey (Ramanujam 1994). Reddy and Reddy (2008) analyzed 11 honey samples of *A. cerana* for pollen spectra and found that most of them were unifloral (10) and only one sample was multifloral. Thirty-two different pollen types belonging to 20 families were recorded. Twenty-one plants recorded from the honey samples were found to be medicinal plants in folklore and tribal medicine. The flower nectar was found to contain Alkaloid and Phenolic compounds (Baker 1977). While foraging on these flowering plants, bees gather the honey mixed with these chemical compounds. Hence, the honey would also have the medicinal property. In folklore medicine, this honey is used for controlling various diseases. According to Reddy and Reddy (2008) Winter samples showed the presence of pollen referable to *Melilotus alba* (Fabaceae), *Evolvulus alsinoides* (Convolvulaceae), *Mimosa hamata* (Mimosaceae), *Feronia elephantum* (Rutaceae), *Sesamum indicum* (Pedaliaceae), *Zizyphus mauritiana* (Rhamnaceae), *Borassus flabellifer* (Aricaceae), *Erythrina indica* (Fabaceae), *Ageratum conyzoides* (Asteraceae), *Crotalaria juncea* (Fabaceae), *Capsicum frutescens* (Solanaceae), *Xanthium strumarium* (Asteraceae), *Alternanthera sessilis* (Amaranthaceae), *Amaranthus viridis* (Amaranthaceae), *Coccinia indica* (Cucurbitaceae), *Celosia argentea* (Amaranthaceae), *Aspidopteris indica*, *Psidium guajava* (Myrtaceae). Of these, the pollen of *Melilotus alba* (47 %), *Ageratum conyzoides* (57.5 %), *Aspidopteris indica* (82.5 %) being represented by more than 45 % and referred as predominant pollen type. Further, these honeys are known as *Melilotus*, *Aspidopteris*, *Ageratum* honeys. The pollen of *Crotalaria juncea* (20 %) represents the secondary pollen type. Other pollen types are placed under important minor and minor pollen categories.

Summer honeys consisted of number of pollen types referable to *Phoenix sylvestris*, *Capsicum frutescens*, *Borassus flabellifer*, *Coccinia indica*, *Tridax procumbens*, *Capparis grandis*, *Dillenia pentagyna*, *Syzygium cumini*, *Chrozophora indica*, *Schleichera oleosa*, *Terminalia arjuna*, *Acacia nilotica*, *Lagerstroemia parviflora*, *Zizyphus xylocarpa*, *Sapindus emarginatus*, *Gardenia lucida*, *Guazuma ulmifolia*, *Madhuca indica*, *Bombax ceiba*, *Feronia elephantum*, *Strychnos potatorum*, *Croton bonplandianum*, *Azadirachta indica*. Of these, the pollen of *Phoenix sylvestris* (53.75–83.75 %), *Dillenia pentagyna* (90 %), *Schleichera oleosa* (53.75 %),

Gardenia lucida (47.5 %) being represented by more than 45 % of the palynoassemblage of the summer honey samples are represented to the predominant pollen types. These honeys are designated as the Phoenix, Dillenia, Schleicheria, Gardenia honeys.

Shubharani et al. (2012) found that the result of microscopic analysis of honey samples from the study area suggested that Coorg region has rich and diversified natural flora and cultivated crops. Among the reported taxa, there is a dominance of tree species (53.84 %) which includes *Eucalyptus* sp., *Jacaranda* sp., *Tecoma stans*, *Semicarpus anacardium*, *Terminalia* sp., *Scheffleria* sp., *Casuarina equisetifolia*, *Acacia* sp., *Albizia lebbek*, *Butea monosperma*, *Dalbergia sissoo*, *Sapindus laurifolia*, *Pongamia pinnata*, *Samanea saman*, *Santalum album*, *Gossypium* sp., *Bombax malabaricum* etc. and with sparse distribution of herbs (24.17 %) and shrubs (20.87 %). The identified honey plants are categorized according to their economic importance like medicines, spices, timber, vegetables, cereals, fibres, fruits and nuts, weeds and ornamental plants (Fig. 12.1). The most preferred and highest contribution of nectar and pollen source for honey bees in the study area belong to the Fabaceae and Asteraceae.

From the above observations, it is clear that the study area exhibits diversified flora and unlimited potential for bee forage, which constitute a valuable source of pollen and nectar crop for honey bees which in turn are important for the survival of bee colonies and also necessary for organized apiculture industry. Moreover, honeybees being the important pollinators contribute to the increased production and yield of commercial, medicinal, and economically viable plants and thereby contribute towards improving rural and forest economy. The result of our study support the views expressed by Zamarlicki (1984) who reported that knowledge of honey plants is the most important factor in bee management and survival of honey bees. Thus, identification of bee flora including their abundance, distribution and floristic information is essential for good yield of honey (Sivaram 1995).

The study suggests and recommends to agencies like forest and rural development departments that it should make all efforts to include the bee forage plants species, particularly tree species in afforestation and social forestry development programmes in Coorg district. The conservation of honey plants are of utmost importance for the development of both natural and wild bee colonies in the region for sustainable environment and maintaining the natural habitat. The decline in bees adversely affects the pollination of flowering plants which in turn results in substantial decrease in the production of agricultural and horticultural crops and economy of the region (Sharma 1972).

12.15 Conclusion

What is described is only a general feature of the major geographical areas in India which can be used as bee pasturage. However, a bee keeper has to build up from this general understanding a more practical and specific understanding of his area as a bee pasturage. In order to get the benefits of bee pollination, the farmers have to

provide for bee forage when there are no crops flowering in the farms. Various trees and shrubs can be planted along hedges, borders, bunds and waste lands, which can provide nectar and pollens to bees. In addition, the farmers can also introduce several crops like coriander, maize, etc. in crop rotation schedules. These crops provide valuable bee forage when needed and at the same time augment the income of the farmers through their produce. The programmes of afforestation, social and farm forestry assume special significance in the national rural development programmes. Afforestation of plants useful to honeybees helps beekeeping. This also helps to develop forests as an ecologically balanced biological unit. A systematic programme of reforestation with bee plants can, in a few years' time, sustain a large number of bee colonies which can also provide gainful self-employment to the local forest or tribal population. The benefits of such a programme will start accruing after five to ten years. In view of this, it is necessary to take up large scale afforestation programmes as soon as possible. Generally under different plantation programmes, the fast growing tree species are selected for plantation. These species have limited use, such as timber or fire wood. To improve the aesthetic appeal and maintain a healthy atmosphere, it is necessary to cultivate and conserve plant species which have multiple uses like timber, fruit, medicine and fuel in addition to their utility to bees. This can allow undertaking of beekeeping for honey production and increased crop production through bee pollination. Keeping in view the above needs, the Central Bee Research Institute has prepared list of bee plants useful for various purposes.

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Chapter 13

Pollination Biology

13.1 Importance of Pollination

The pollination of flowering plants is a critical ecological “service” in both natural and agricultural ecosystems. Pollination is not replaceable by technology, and thus is of astronomical value economically as well as ethically and aesthetically. The majority of angiosperm species—by some estimates up to 90 %—rely in whole or in part on animals for pollination. Pollinators provide an essential ecosystem service to both natural and agricultural ecosystems.

Animal pollination plays an important role in the reproduction and fruit set of many cultivated, flowering crop plants and wild plant communities. Bees comprise an estimated 25,000–30,000 species worldwide, all obligate flower visitors. Animal pollination is effected by many different species ranging from vertebrates (e.g., bats) to invertebrates such as insects and intensity or quality of pollination may be affected if pollinator species change. Introduction of non-native (exotic) pollinators might have an impact on both native plants and pollinator communities. Thus, the introduction of non-native bees may cause direct and indirect ecological impacts.

The flowering plants (angiosperms) comprise approximately one-sixth of the total number of described species (250,000 species) and insects about two-thirds. These groups thus dominate the flora and fauna of Earth’s terrestrial habitats, and interactions between them are dominant components of all terrestrial ecosystems (Buchmann and Nabhan 1996). One of the most ecologically important of these interactions is that between flowering plants and pollinator insects (Klein et al. 2007). Most of these flowering plants in some studies estimates are as high as 90 % (Kearns et al. 1998) including many important agricultural species, which are pollinated by animals, mainly insects (Daily 1997); the rest of the angiosperms rely on abiotic agents such as wind or water. Animal pollination plays an important role in the reproduction and fruit set of many cultivated, flowering crop plants (Nabhan and Buchmann 1997; Kearns et al. 1998; Westerkamp and Gottsberger 2000) and wild plant communities (Kearns and Inouye 1997; Larson and Barrett 2000; Ashman et al. 2004; Kremen et al. 2007). It contributes to the maintenance of plant diversity, in terms of species number, genetic variation and richness of functional groups (Fontaine et al. 2005; Ashworth et al. 2009).

Flowering plants form a mutualistic relationship with their flower-visiting pollinators. Mutualisms are defined as interspecific interactions between two participants, in which partners gain a net benefit (Bronstein 1994). A competition interaction is one of the well-known instances of ecological and evolutionary consequences of species diversity within mutualistic interactions (Jason and Bruna 2000; Stanton 2003; Palmer et al. 2003). When mutualists share the same resource, competition for access to the resources or services its partners provide may be frequent (e.g., competition between pollinators for floral resources) and is important for understanding the mechanisms underlying host use by multiple species (Palmer et al. 2003).

Pollination is defined as the transfer of pollen from the anther (the male part of a flower) to a stigma (female part of a flower) of the same or different flower, thus enabling fertilization to take place (Lovatt 1997). Self-pollination occurs when the anther and stigma are from the same flower, from different flowers on the same plant, or from flowers on different plants of the same cultivar (Mauseth 2009). Cross-pollination is the transfer of pollen from one cultivar to the flower of a different cultivar of the same species. Bees are the main pollinating group in many climate zones and in most geographic regions (Michener 2000). Bees comprise an estimated 25,000–30,000 species worldwide, all obligate flower visitors. Adding these species to other obligate or facultative pollinators such as flies, butterflies and moths, beetles, and birds, the total number of flower-visiting species worldwide is estimated to be nearly 300,000 (Kearns et al. 1998).

Pollinators are one of the important ecosystem elements and are well known to provide key ecosystem services, specifically pollination, to both natural and agro-ecosystems. An ecosystem is a unit of interdependent organisms that interact with each other and with abiotic factors. Ecosystems are considered functional groups composed of elements (structures) and processes (functions). The ecosystem structures are the biotic components (biological species), which can be organized according to the functions they have in the system (i.e., their trophic level). The ecosystem processes, or functions, refers to mechanistic processes such as decomposition, productivity and nitrogen fixation (De Marco and Coelho 2004). Ecosystem services are natural functions that benefit human populations (Daily 1999). These services include soil formation, nutrient cycling, gas regulation, climate regulation, biological control pollination as well as recreation and cultural. Hence, understanding the interaction of pollinators is important to improve our understanding of ecosystem services and functions.

Approximately 80 % of all flowering plant species are specialized for pollination by animals, mostly insects. The negative impact of the loss of pollinators is strongly felt in agricultural biodiversity. The role of pollinators is, among other things, to ensure reproduction, fruit set development, and dispersal in plants, both in agro-ecosystems and natural ecosystems. In turn, plants need to exist in order for pollinators to be able to feed. Indeed, some plant species rely upon a few types of pollinators to provide pollination services. Some pollinators such as bees also provide food and additional income for rural families, in the form of honey and other byproducts—thus, declining pollinator populations impact on the sustainable livelihoods of rural families. A decline in pollinator populations also affects plant

Table 13.1 Crops that are dependent on or benefit from honeybee pollination. (Partap 2011)

Crops dependent on bee pollination	Crops benefitting from bee pollination
<i>Fruit and nut crops</i>	
Almond	Apricot (few varieties)
Avocado	Blackberry
Apple (all commercial varieties)	Citrus
Apricot (some varieties)	Peach
Cherry (many varieties)	Persimmon
Kiwi fruit	Strawberry
Litchi	
Mango	
Plum (many varieties)	
Pear (many varieties)	
<i>Vegetable seed crops</i>	
Cabbage	Beans
Carrot	Capscium
Cauliflower	Eggplant
Cucumber	Okra
Onion	Tomato
Pumpkin	
Radish	
Sqash	
Turnip	
<i>Vegetable crops</i>	
Bitter gourd	
Bottle gourd	
Muskmelon	
Pumpkin	
Sponge gourd	
Squash	
Watermelon	
<i>Oilseed crops</i>	
Sunflower	Mustard
Niger	Rape
	Cotton
<i>Spice crops</i>	
	Greater cardamom
	Chillies
	Coriander

biodiversity. Indigenous species particularly have been subject to external pressures such as habitat destruction and fragmentation resulting from activities such as land clearing for agricultural purposes, pesticide use, tourism, and the introduction of exotic species.

Pollinators such as bees, birds, and bats affect 35 % of the world's crop production (Table 13.1). Animal pollinators increase the outputs of 87 of the leading food crops worldwide. In the continents of Latin America, Africa, and Asia, an average of 40 % of the land area of crops is planted to crops with some dependence on animal pollinators. These are low estimates, as they do not include secondary crops, medicinal plants

or wild-harvested crops, but they do provide an indication of the extent to which pollinators are essential for many “diversities”: diversity in diet, biological diversity including its agricultural dimension and the maintenance of a diverse and resilient natural resource base.

Growing evidences suggests that healthy pollination services are threatened in many parts of the world. A decline in pollinator populations also affects plant biodiversity. Native pollinator species may decrease when their nesting habitats are destroyed, when they find less wild flowering plants to forage on throughout their life cycle, and when they are impacted by injudicious use of pesticides. At least one-third of the world’s agricultural crops depend upon pollination provided by insects and other animals. As farm fields have become larger, and the use of agricultural chemicals increases, mounting evidence points to a potentially serious decline in pollinators.

13.2 Importance of Pollination to Agriculture and Biodiversity

As explained, pollination is vital in completing the life cycles of plants. It ensures better crop yields whether in grain crops, seeds, or fruit crops and is also necessary for the maintenance of biodiversity. It helps in soil conservation and soil fertility improvement through enhancing replenishment of soil nutrients, thus helping in conservation of environment and biodiversity, improving livelihoods through enhancing agricultural production.

13.3 Honeybees as Pollinators

There are about 25,000 species of bees found in the world. They include honeybees, bumble bees, stingless bees, and solitary bees. Bees are the most efficient and most important pollinators of many cultivated crops and wild flora. A large number of bees on a crop ensure good pollination that result in higher yields and better quality produce. Bees visiting the flowers of a crop become conditioned to that particular crop and visit a number of its flowers during single foraging trips. Further, while collecting nectar and pollen, the bee brushes against the anthers of a flower and some pollen grains are picked up by the hairs on its body and head. When the bee visits another flower, some of the pollen grains are captured by the sticky surface of a receptive stigma, thus effecting cross-pollination. Bees have the following characteristics that make them the most important pollinators:

1. Bees are social insects; while other insects collect nectar mostly to satisfy their individual needs, bees collect nectar and pollen to feed their young.
2. Bees have body hairs. When a bee visits a flower, some pollen becomes attached to its body and is transferred to the stigma of another flower that the bee visits next, thus accomplishing pollination.

3. Bees show flower constancy: e.g., a foraging bee usually moves from one flower to another of the same species for as long as nectar and pollen are available. Other insects haphazardly visit flowers of different species. This constancy in foraging is important for effective cross-pollination of a particular plant species.
4. Many species of bees, e.g., honeybees and stingless bees, are kept in man-made nests and mass-reared for honey production.
5. Many species of bees can also be managed for crop pollination. The most important are honeybees, bumble bees, and solitary bees; e.g., alkali bees, hornfaced bees, and leaf-cutter bees. Honeybees are the most efficient of all the bees as pollinators of crops and natural flora. This is because their body parts are especially modified to pick up pollen grains; they have the potential to work for long hours, show flower constancy, and are adapted to different climates (McGregor 1976; Free 1993). Most importantly, some honeybee species can be managed and transported to fields to pollinate crops. Moreover, technology for managing them for production of honey and pollination is available. In addition to this, Free (1993) lists the following characteristics—social and behavioral—that make honeybees the most effective and reliable crop pollinators.
 - (a) Honeybees live in colonies where the young are nursed and fed by adults with a mixture of honey and pollen throughout the year. They work together to supply each other's needs and cooperate to raise offspring. Honeybee colonies are large compared to other bees consisting of 5,000–80,000 individuals depending upon the species.
 - (b) They have the potential for long working hours. They start their foraging early in the morning and cease late in the evening, working many hours a day.
 - (c) Honeybees have evolved a special communication system by which thousands of foragers can be deployed when a good food source is present.
 - (d) They are micromanipulators of flowers and visit many flowers per unit time compared to other bee species.
 - (e) Some species can be managed in large numbers and moved to crops where and when necessary.
 - (f) Most importantly, honeybees provide honey, beeswax, and other bee products.
 - (g) Finally, they are found in different geographical areas and are adapted to different altitudes and climates.

13.4 Honeybee Species in Asia

Nine species of honeybees, including the giant honeybees or rock bees (*Apis dorsata* and *Apis laboriosa*), the little honeybee (*Apis florea*), the small dark honeybees (*Apis andreniformis*), the Asian honeybee (*Apis cerana*), and the European honeybee (*Apis mellifera*), are found in Asia. Among these, *A. dorsata*, *A. laboriosa*, *A. florea* and *A. andreniformis* cannot be kept in hives. Honey from these bees is harvested by traditional honey-hunting methods. Other bees, *A. cerana* and *A. mellifera*, are being managed in hives for honey production and pollination.

A. dorsata is found throughout the Asian region up to 2,000 m. It builds single comb nests in the open, on branches of tall trees and tall buildings and chimneys, in shady places during summer and sunny places during winter. As many as 70 or more colonies can be found on a single tree. This species is migratory in nature; a colony never stays in the same place for more than 6 months. *A. dorsata* produces harvestable amounts of honey and is an important pollinator of many crops and other plants. It nests in low hill areas during winter and migrates to the high hills in summer. *A. laboriosa* is found from 1,200 to 3,500 m in remote mountainous areas of Bhutan, China, India, and Nepal (Sakagami et al. 1980; Underwood 1986; Batra 1995). It nests beneath rock overhangs on vertical cliff faces. Colonies are found at a height of at least 10 m above the ground and occur in groups. Like *A. dorsata*, 70 or more colonies can be found at a single cliff site. It is also an important crop pollinator. It is also migratory in nature and a colony does not remain in one place the year round. *A. florea* is one of the smallest honeybee species and is called the dwarf bee. It also builds single comb nests on branches of bushes, hedges, small trees, and chimneys, etc. This species is found in the plains and in hilly areas up to 500 m.

It is also migratory in nature and a colony seldom stays in one place for more than 6 months. *A. florea* is another pollinator of agricultural crops. *A. andreniformis* is found in the tropical region in the south of Hengduan mountain range of China, Thailand, and Malaysia where the elevation is lower than 1,000 m. It builds its nests in small trees or bushes. Concrete information on this bee species is very scarce (cf. Chap. 20). Formerly in taxonomy, it was considered as a subspecies of *A. florea* until 1986 when Wu and Kuang (1987) convincingly identified it as a separate species. This bee was first named “*MicrApis andreniformis*” and migrates twice a year, in February to March and August to September. Generally, *A. andreniformis* is regarded as a species on a relatively low phylogenetic level within the genus (Wu and Kuang 1987).

The Asian honeybee, *A. cerana*, and the European honeybee, *A. mellifera*, can be kept in hives and managed for honey production and crop pollination. They are cavity-nesting, hive bees. *A. cerana*, the Asian hive bee or Himalayan hive bee, is widespread up to 3,000 m throughout Asia. It has a gentle temperament, an industrious nature, and good hygiene qualities (Verma 1990). Unlike *A. dorsata*, *A. laboriosa*, *A. andreniformis*, and *A. florea* that build single comb nests in the open, *A. cerana* makes multiple parallel combs inside a cavity. Beekeeping with this bee is a common tradition among several countries of Asia. Farmers keep it in traditional fixed-comb hives such as log, wall, and earthen-pitcher hives and in movable-frame wooden hives. A colony of *A. cerana* produces 5–20 kg of honey per year and is an excellent crop pollinator. This species has not become popular among commercial beekeepers because of its relatively low honey production and undesirable behavioral traits such as frequent swarming, absconding, and robbing habits. *A. mellifera* has been imported to Asia for commercial honey production. This species is kept in hives and makes parallel combs. It has gained high popularity among commercial beekeepers because it produces more honey than *A. cerana*, maintains a prolific queen, has low swarming and absconding tendencies and has good honey-gathering qualities. However, beekeeping with this species requires expensive technology and

a high degree of chemical treatment to control diseases and parasites to which it is more susceptible (Verma 1990; Ahmad et al. 2002). Recently, more species of *Apis* have been identified in Asia. These include *Apis koschevnikovi* (also named for a short period of time as *Apis vechti*) reported from Sabah, Malaysia, *Apis nigrocincta* reported from Philippines and *Apis binghami* reported from Sulawesi islands of Indonesia.

13.5 Role of Asian Honeybees in Crop Pollination

It has been estimated that the Himalayan region has over six million colonies and nests of indigenous and exotic honeybees that produce over 76,000 metric tonnes of honey every year. The figures on the number of honeybee colonies and honey production for Asia would obviously be very high. The honeybees play an important role in the pollination of various crops and natural flora and enhance vibrancy of floral diversity and agricultural productivity, thus contributing to the income and livelihood of poor mountain communities. A number of crops such as vegetable, fruit and nuts, oilseed, spices and forage and fibre crops either depend on or are benefited by honeybee pollination (Table 13.1).

13.6 Pollination Efficiency

The pollination efficiency of different insects has been evaluated on the basis of a number of characteristics. It has been recognised that there are inherent differences in the ability of various species to effect pollination in spite of their abundance. Normally, the efficiency of an insect species as a pollinator has been attributed to its foraging behavior and the amount of loose pollen grains adhering to its body (Bohart and Nye 1960; Free 1993). Pollination indices are calculated on the basis of relative abundance and foraging behavior (Sharma 1990; Partap and Verma 1992, 1994; Verma and Partap 1993, 1994). Foraging efficiency of honeybees depends upon their foraging behavior, for example, the duration of foraging period, time spent on the flower, and number of flowers visited per minute (i.e., foraging speed and foraging rate), whether foraging as a top-worker or side-worker and preferences for pollen or nectar and the amount of loose pollen carried on the body. Foraging speed (time spent per flower) and foraging rate (number of flowers visited per minute) depend upon the foraging behavior of insects and floral structure of the crop concerned, particularly the corolla depth (Gilbert 1980). Free and Williams (1973) reported that honeybees spend 131 s per kale flower when collecting pollen loads and 94 s when collecting nectar only.

The number of loose pollen grains on the body of foragers depends upon the size of the bee and the way it worked on the flowers. Normally, the “top-workers” carry more pollen grains on their bodies than the “side-worker” bees. Bohart and Nye

(1960) reported that pollen collecting honeybees on carrot bloom literally waded across the head, swished their abdomens back and forth, and scrapped the pollen from stamens with their forelegs. The nectar collectors stood higher on the flowers, moved about less, and lapped droplets from the exposed nectaries. Nectar robbing in cauliflower bloom by many honeybee foragers has been found in northwest India by Kumar et al. (1994). Side-foraging of cauliflower nectar by *A. cerana* and *A. mellifera* and of okra nectar by *A. cerana* has been reported by Kapoor and Dhaliwal (1989) and Mishra et al. (1987), respectively. Sihag and Rathi (1994) have also suggested a formula for calculating performance scores of insect visitors on different pollination attributes. In addition to pollinators, pollination of a particular crop or plant species also depends upon its attractiveness and rewards offered to the pollinators.

Asian hive bees, *A. cerana*, have been reported to be more efficient pollinators of various fruit and vegetable crops than European *A. mellifera*. In their field experiments on the foraging behavior of *A. cerana* on cabbage, cauliflowers, broad leaf mustard, and lettuce conducted in the Kathmandu valley of Nepal, Partap and Verma (1992, 1994) and Verma and Partap (1993, 1994) reported that the foragers started working on cauliflower and cabbage bloom early in the morning and ceased late in the evening, thus pollinating these crops for comparatively longer hours than *A. mellifera*.

Similar results were obtained by Verma and Rana (1994) on apple crop in the Shimla hills of the northwestern Indian Himalayas. Further, in *A. cerana*, pollen collectors outnumbered nectar collectors, while in *A. mellifera*, nectar collectors outnumbered pollen collectors, though the ratio between pollen and nectar collectors decreased during the day for both species on broad leaf mustard, cabbage, cauliflower and lettuce in Kathmandu valley of Nepal (Verma and Partap 1993).

Regarding the foraging behavior of *A. cerana* on lettuce and radish, the bees started their foraging activities early in the morning and ceased late in the evening; foraging for a period of 11.50 ± 0.04 h/day on radish and 3.00 ± 0.05 h/day on lettuce. The peak foraging hours occurred from 11:00 to 13:00 for radish and 9:00 to 11:00 for lettuce crop. The average duration of a foraging trip was 22.1 ± 0.03 min for radish and 15.66 ± 0.04 min for lettuce. Furthermore, the bees visited an average of eight, nine and five flowers per minute of radish during different hours of the day and ten flowers per minute of lettuce during morning hours (Verma and Partap 1993; Partap and Verma 1994).

A. cerana worked on the flowers mainly from the top position as “top workers”, while *A. mellifera* mostly from the side position “side-workers” in cabbage, cauliflowers and radish crops in Kathmandu valley of Nepal (Partap and Verma 1992, 1994; Verma and Partap 1993, 1994). According to Erickson and Peterson (1979a, b), 14,666 and 9,582 carrot pollen grains were found on the body of pollen gatherers and nectar gatherers, respectively. Priti and Sihag (1997) found that on cauliflower bloom, *A. dorsata* carried the maximum and *Musca domestica* the minimum number of loose pollen grains. Pollen grain carrying capacity varied in different foraging groups of honeybees and also on different crops. Different workers have reported different results, for example, Kumar et al. (1993) found that nectar gatherers carried pollen grains either equal to or greater than pollen gatherers on toria and cauliflower.

Other workers have found more pollen grains over the body of pollen gatherers than nectar gatherers on cauliflower (Dhaliwal 1980; Kapoor 1983) and onion (Kumar et al. 1985).

13.7 Honeybees as Pollinators of Various Crops

A great deal of experimental research on insect pollination of various crops has been carried out in different parts of the world, especially on type (species) and number of insect visitors, their foraging behavior on the flowers and their impact on the yield and quality of various agricultural and horticultural crops. The pollination literature is full of such references (McGregor 1976; Free 1993; Kevan 1995). However, most of the research has been done in developed countries of the world where honeybees are being used for the pollination of various crops. The two notable reference books by McGregor (1976) and by Free (1993) present excellent details of research undertaken worldwide on insect pollination of crops. Most references speak about the role and importance of different species of honeybees in pollinating various crops.

The role of honeybees in pollination of several other crops has also been studied by various scientists in different parts of the world. For example, in Egypt, honeybees are reported as the most important pollinators of *Brassica oleracea* comprising 98 % of visitors (Hussein and Abdel-Aal 1982). Similarly, in New Zealand, honeybees comprised 99 % of all flower visitors of cauliflower (Forster et al. 1973). In the Indian subcontinent, *A. cerana*, *A. dorsata*, and *A. florea* are all important in pollinating cauliflower, although to various extents in different localities. Thirty-four species of insects, including honeybees, visited the cauliflower bloom at Solan, Himachal Pradesh in northwestern India (Sharma et al. 1974). *A. cerana* has been reported as one of the most frequent visitors of nine cultivars of okra in northwestern Indian Himalayas (Mishra et al. 1987) and onion bloom in the United States (Treherne 1923; Trofimec 1940; Agati 1952; Bohart et al. 1970; Caron et al. 1975). In India, *A. cerana*, *A. dorsata*, *A. florea*, and *Trigona iridipennis* were reported as primary insect pollinators of onion (Singh and Dharmwal 1970; Jadhav 1981; Rao and Suryanarayana 1989). Kumar et al. (1985) found that three onion species, *Allium cepa*, *Allium fistulosum*, and *Allium cepafistulosum*, greatly benefited by insect pollination, especially by *A. cerana* and flies. Carrot flowers are visited by different types of insects (Bohart and Nye 1960); however, pollen gathering honeybees were more valuable than nectar gatherers.

13.8 Impact on Crop Yield and Quality

Several studies were specifically undertaken to show the impact of honeybees, particularly *A. cerana*, in enhancing the productivity levels of various crops such as fruit and nuts, vegetables, pulses, oilseeds, spices and fibre, and forage crops (Deodikar and Suryanarayana 1977; Dulta and Verma 1987; Gupta et al. 1993, 2000;

Table 13.2 Impact of *Apis cerana* pollination on different crops. (Crane 1991)

Fruit set		Seed		Seed for propagation	
Crop	Increase	Crop	Increase	Crop	Increase
Apple	× 24	Cardomom	× 6	Berseem	× 2.7
Cranberry	× 5	Cardomom, greater c.	× 10	Cauliflower	× 1.2
Lemon	× 15–17	Horsegram	× 6	Onion	× 1.7
Loquat	× 3	Safflower	× 2.2	Radish	× 1.2
Lychee	× 2	Sarson	× 1.4–1.6		
Peach	× 2	Sesame	× 1.3		
Pear	× 14	Sunflower	× 1.5		
Persimmon	× 1.2				
Plum	× 6				
Watermelon	× 1.6				

Partap 2000a, b; Partap and Verma 1992, 1994; Partap et al. 2000a, b; Singh et al. 2000; Verma and Partap 1993, 1994). These studies have shown that pollination by *A. cerana* increases fruit and seed set, enhances quality of fruit (shape, size, weight, colour, and taste) and seeds, and reduces premature fruit drop. The quality of pollination is determined by the number of colonies per unit area, the strength of bee colonies, placement of colonies in the field, the time of placement of bee colonies, and the weather conditions. Experiences from pilot experiments have shown that the best results are achieved by placing strong bee colonies free of diseases with large amounts of unsealed brood when the crop is at 5–10 % flowering (Free 1993; Verma and Partap 1993).

Research conducted in some countries of the Asian region has proved that honeybee pollination increases the yield and quality of apple in Shimla hills of Himachal Pradesh in Northwestern India (Dulta and Verma 1987; Gupta et al. 1993), peach, plum, citrus, and strawberry in Kathmandu valley of Nepal (Partap 2000a, b; Partap et al. 2000a, b), and kiwi in Shimla hills of Himachal Pradesh, India (Gupta et al. 2000; Table 13.2). The results of this research also showed that honeybee pollination not only increased the fruit set but also reduced fruit drop in apple, peach, plum, and citrus. Results further showed an increase in the fruit juice and sugar content in citrus and reduction in the percentage of misshapen fruits in strawberry (Table 13.2). Looking at Table 13.2, we find an increase of 10 % in the apple fruit set while in the case of strawberries; it is 112 % with a fruit weight increase of 33–48 %, respectively, showing benefits of perfected pollination by honeybees under experimental conditions at the micro level.

Studies conducted in the Kathmandu valley of Nepal have also shown that honeybee pollination enhanced seed production and quality of seed in various vegetable crops such as cabbage, cauliflower, radish, broad leaf mustard, and lettuce (Partap and Verma 1992, 1994; Verma and Partap 1993, 1994). These crops, when pollinated by *A. cerana*, produced more and heavier seeds that showed a higher percentage of germination than the control plants. These results confirm the usefulness of bee pollination and its role in increasing crop productivity and improving the quality of fruit and seeds (Table 13.3). Scientific evidence from other parts of India confirms

Table 13.3 Impact of *Apis cerana* pollination on productivity of different crops. (EdelGive Foundation© Under The Mango Tree 2011)

Crop	Increase in crop productivity (%)
Capsicum	227.05
Tomato	160.61
Cashew	157.89
Pigeon pea	133.33
Flat bean	128.57
Chick pea	79.5
Mustard	75.00
Mango	68.42
Banana	63.16
Niger	60.00
Papaya	60.00
French bean	41.15
Jowar	33.33
Brinjal	31.25
Ridge gourd	27.27
Bitter gourd	– 21.52

that bee pollination improves the yield and quality of other vegetable crops such as asparagus, carrots, onion, turnips, and several other crops (Deodikar and Suryanarayana 1977). Honeybee pollination has been reported to increase the productivity of oil rape seeds by 18.7–37.8 % and oil extraction could be increased up to 10.4 % in Manipur, India (Singh et al. 2000). Production was increased by 34.5–36.4 % in sunflower and 38.5–43.6 % in case of radish seed production (Verma and Partap 1993; Partap and Verma 1994; Singh et al. 2000). In other crops, such as buckwheat, soybean, and cotton, the production increased from 23.8 to 77.7 % (Kozin 1976).

Researchers in different parts of the world reported different percentages of increase in seed set due to pollinators. Increases of 22–100 %, 100–300 %, 100–125 %, 91–135.4 %, and 353.5–987.8 % in the seed yield have been reported due to assured pollination by bees in radish, cabbage, turnip, carrot, and onion crops, respectively (Singh 1997). The results of such studies on important vegetable crops are given below. An increase of 300 % in seed crops of cabbage was reported due to insect pollination by Radchenko (1966). Even greater effects of insect pollination have been reported from India, Pakistan, and Bangladesh. Rauala (1972) reported 68 and 9 % set in cages with and without honeybees, respectively. Increased seed set (129 %) was found, due to presence of honeybees, by Muhammad et al. (1973). Sihag (1986) found that plants caged to exclude insects and plants not caged produced 13 and 978 pods per plant, respectively. Similarly, 9.0 and 11.5 seeds per pod in caged (without insects) and open plots were found by Alam et al. (1987). Tewari and Singh (1983) demonstrated a decrease in seed set and yield with increasing distance from three *A. cerana* colonies. Kumar et al. (1989) found significantly higher seed set and seed weight in open-pollinated than in bagged flowers in five cultivars of cauliflower.

Insect pollination increased the number of seeds in a pod (Free 1976) and there was a positive correlation between the number of seeds in a pod and its weight. Tanda (1984, 1985) reported a 19 % increase in okra yield in Punjab, India, due to intensive bee pollination. Mishra et al. (1987) found that the weight and length of

capsules, and seed number per capsule, were significantly higher in open-pollinated than in bagged flowers of okra. In Poland, Woyke (1981) found that onion plots caged without bees, caged with bees, and not caged produced 2, 210, and 669 seeds per head, respectively. Also, in India, Kumar et al. (1989) found greater set and yield and better germination of onion seeds from plots caged with bees than from plots caged without bees and open plots (61, 90, 72 %) and estimated seed yields of 73, 275, 97 kg/ha, respectively, on plots caged without bees, caged with bees and not caged. Rao and Suryanarayana (1989) also reported higher seed yield in onion due to placement of bee colonies.

Honeybees are a subdivision of bees which is mainly distinguished by the manufacture and storage of honey and the construction of persistent, colonial nests out of wax. Honeybees are the members of the tribe Apini, in the genus *Apis*. India is a tropical country bestowed with highly diversified ecosystems. Varied ecological conditions with diversified flora have provided favorable habitat for various honeybee species in India. The giant honeybee (*Apis dorsata* F.), the oriental hive bee (*A. cerana* F.), dwarf bee (*A. florea* F.), and several species of stingless bees (*Trigona* and *Melipona*) are widely distributed in India. These species pollinate various plants (Bright et al. 1998), and produce hive products such as honey, wax, pollen, etc. which are useful to mankind (Shukla and Upadhyay 2007).

A. cerana is widespread in temperate and tropical Asia. There are many different subspecies and races of *A. cerana*, due to wide range of habitats it occupies from temperate mountain regions to tropical islands. In his 1988 monograph, Ruttner summarized the data on morphometric variation in *A. cerana*. He recognized four subspecies of *A. cerana* as follows: *A. cerana cerana* in northern Asia; *A. cerana indica* in southern Asia, *A. cerana japonica* in Japan; and *A. cerana himalaya* in the Himalayan region. Other honeybee species in Asia showing behavior similar to *A. cerana* are *A. koschevnikovi*, *A. nigrocincta*, and *A. cerana nuluensis*. These gentle species of bees have long been managed as useful honeybees in many parts of Asia and their honey and wax valued.

It is commonly believed that nearly 70 % of cultivated crops all over the world are cross-pollinated and depend on insects like honeybees for pollination. Dwindling population of such useful pollinating insects has now become a global problem. The importance of bees is often underlined with their role in pollination services and income generation by production of honey and there has been a common concern that population of indigenous bees is declining at an alarming rate. In the study site, beekeeping has just been initiated with indigenous honeybee *A. cerana*.

Honeybees as pollinators are well-known to enhance crop productivity of cross-pollinated crops. Natural pollination through wild honeybees is normally occurring in crop fields. However, pollination activity through apiculture enhances both quality and productivity of various crops. India introduced *Apis mellifera* in the north Indian states in the early 1970s to enhance crop production in apple, kinnow, orange, and vegetables such as cucurbits, etc. In eastern India, this species is extensively used in enhancing litchi production. Standardization of colony requirement for these crops has enabled commercial beekeeping with custom hiring in various states. In other parts of the country, the dominant commercial exploitation is by using *Apis cerana indica*. The pollination induced through pollinators through honeybees help in early

setting of seeds, resulting in early and more uniform crop yield. It is estimated that about 5–25 % increase in yields of various crops is due to pollination by honeybees, and in crops like apple in absence of bees no yield is expected. The most important crops where substantial increases in yields can be obtained are litchi, almond, citrus, grape, cucurbits, plum, pear, cashew, papaya, and cardamom.

Honeybee could also be used as an effective tool for increasing production and income of farmers. *Apis cerana* was used in the past, but *Apis mellifera* introduced and domesticated, provides pollination and gives yields up to 40–50 kg honey per box. Various products, viz. bee wax, bee venom, royal jelly etc. could also be prepared. At present, there are about one million colonies of honeybees in India and the National Commission on Agriculture has estimated that given the existing vegetational wealth, 150 million bee colonies can be sustained which would be capable of producing 1.5 lakh tonnes of honey. At the same time, it generates employment for 15 million rural and tribal families besides phenomenal improvement in crop productivity and higher returns from unit area.

13.9 Role of *Apis cerana* in Crop Pollination

Honeybees present an uncommon opportunity for diversification in agriculture. Crop pollination is an essential ecosystem service, which is efficiently provided by different pollinators. Amongst various pollinators available, Dyer and Seeley (1991b) reported that *Apis cerana* shows a disproportionately high mass-specific metabolic rate, their foragers make many more trips per day in the same habitat than do foragers of the other species. *Apis cerana* can therefore be considered as one of the most efficient pollinators. In India, crop pollination has been negatively affected due to reduction in density and population of efficient pollinators. With the help of beekeeping with indigenous honeybee, *Apis cerana*, its density and availability as a pollinator have increased in some areas. The results suggest that increasing number of *A. cerana indica* through beekeeping at different scales would help to ensure higher crop yields. Adequate number of *A. cerana indica* is observed to be vital for significant crop production. More widely accepted is that *A. cerana* does well in disturbed or extensively modified habitats. For example in Hong Kong, *A. cerana* visits 86 % of plant species and pollinates so successfully as to maintain that island's diverse flora (Corlett 2001 cited in Oldroyd and Wongsiri 2006). Of relevance to Australian environments, the Indica genotype of *Apis cerana* in Sri Lanka was observed foraging on *Eucalyptus robusta*: myrtaceae (River red gum) and it is cited as being an excellent source of honey (Punchihewa 1994).

The range of *Apis cerana*, the Asiatic honeybee, extends from tropical Asia across China as far as Siberia. The vast natural distribution and diverse climatic zones provide this honeybee with associations with a diverse flora of wild plants and cultivated crops from tropical, sub-tropical and temperate regions and from the plains and hills of each region. A large body of literature is available on *Apis mellifera* and its relationship with temperate crops (Free 1970; McGregor 1976; Table 13.1).

13.10 Role of *Apis cerana* on Crop Productivity

The list of the crops is endless whose productivity can be increased by introducing hives of *Apis cerana*. The data presented in Table 13.2 shows the effect of hives of *Apis cerana* on fruit set or crop yield of some of the crops. They were obtained in two types of experiment, in which crop yields from plants were compared when (1) some were accessible to bees and other insects, and others protected from them, for instance by bagging, and (2) some were on plots to which hives of *Apis cerana* were taken and to others on plots to which they were not taken. In most studies a commercial yield seemed to depend on pollination by *A. cerana*. Increase in yield or fruit set, due entirely or largely to the presence of *A. cerana*, was quantified for a number of crops (Table 13.2).

In extensive studies with *Apis cerana*, EdelGive Foundation© Under the Mango Tree (2011) found that out of 16 plants studied for the productivity, only one species showed reduced productivity in bee box area (Table 13.3). Fifteen plant species showed considerable increase in productivity in areas with *A. cerana* bee boxes. The exact role of *A. cerana* as a pollinator can only be assessed by in-depth study of their foraging and analysis of nectar and pollen. However, the increase in productivity can still be ascertained to the presence *A. cerana* in good density in areas with boxes. Of the studied plants for productivity, 15 plants showed increased productivity with high margin—the lowest being 27 % and highest being 227 %. Thus the role of *A. cerana* in productivity cannot be questioned and can only be studied in detail further to explore possibilities of deliberate and strategic attempt to restore population of *A. cerana* in the ecosystem, landscape and regional level to meet critical objective like increase in crop production, honey production, improvement in livelihood and ecological balance. This short term study presents a hope that with *Apis cerana* the crop production can be engineered positively.

Very few recommendations have been made as to the number of hives of *A. cerana* to be used per hectare on different crops; one report referred to here suggests 4 hives/ha for cardamom. In general, a hive of *A. cerana* will contain fewer foragers/pollinators than one hive of *A. mellifera*, and the *A. cerana* bees will forage over a smaller area. In India, Dhaliwal and Sharma (1973) found that *A. cerana* foraged within a range of about 1000 m from the hive, on a crop of cauliflower. Many figures have been quoted for the range of *A. mellifera*; if we take 1400 m as a modest figure for foraging on a uniform crop, the area foraged by an *A. cerana* colony would be only half that foraged by an *A. mellifera* colony. In principle therefore, on the same crop, extra hives of *A. cerana* would be needed so that bees fly to all parts of it, and it is essential that these hives are at more locations on the crop, closer together, than hives of *A. mellifera*. However, the role of *A. cerana* in pollination has been studied fragmentarily and most of the reports available come from India. Nevertheless, these records show that *A. cerana* is associated with the flowers of all categories of entomophilously pollinated crops:

1. Fruit trees.
2. Vegetables and pulses.
3. Oilseed crops.

Table 13.4 Impact of *Apis cerana* pollination on fruit productivity

Crop	Increase in fruit set (%)	Increase in fruit weight (%)	Increase in fruit size (length/diameter) (%)	References
Apple	10	33	15/10	Dulta and Verma (1987)
Peach	22	44	29/33	Partap et al. (1987)
Plum	13	39	11/14	Partap et al. (2000a, b)
Citrus	24	35	9/35	Partap (2000a)
Strawberry	112	48	Misshapen fruit decreased by 5 %	Partap (2000b)

Also reduced premature fruit drop in apple, peach, plum, and citrus

4. Condiment and spice crops.
5. Fiber crops.
6. Forage and fodder crops.
7. Crops producing dyes.

This chapter attempts to summarize the available information on *A. cerana* and its relationships in pollination and flower visiting with these crop types.

13.10.1 Fruit Trees

The association of *A. cerana* with fruit trees is well established (Table 13.4). The trees for which clear documentation has been made include almond *Prunus dulcis* (Miller) D. A. Webb (Muttoo 1950; Bhalla et al. 1983a), apple *Malus domestica* Borkh (Sharma 1961; Rai and Gupta 1983; Verma and Dutta 1986), lime *Citrus aurantifolia* (Christm.) Swingle (Anonymous 1981), cranberry *Vaccinium macrocarpon* Alt. (Sharma 1961), litchi *Litchi chinensis* Sonner (Pandey and Yadav 1970; Dhaliwal et al. 1977; Phadke and Naim 1974; Dhoble and Shinde 1982), plum *Prunus domestica* L. (Sharma 1961), peach *Prunus persica* (L.) Batsch. (Bhalla et al. 1983b; Kumar et al. 1984), phalsa *Grewia asiatica* L. (Parmar 1976), strawberry *Fragaria vesca* L. (Singh 1979), Chinese jujube *Ziziphus jujuba* Mill. (Ackerman 1961), durian *Durio zibethinus* Murray (Crane and Walker 1983), carambola *Averrhoa carambola* L. (Nand 1971), coconut palm *Cocos nucifera* L. (Anonymous 1982), borassus palm *Borassus flabellifer* L. (Seethalakshmi and Percy 1979), and pear *Pyrus communis* L. (Sharma 1961). Muttoo (1950) cited the lack of fruit set in cultivated almonds resulted from failure in pollination, and he pleaded for the use of honeybees for the production of fruits in India. Sharma (1961) reported that 50–78 % of the insect visitors to pear and cranberry were honeybees and on plum, apple, cherry, and peach the population of honeybees represented 33, 70, 45, and 63 % respectively. *A. cerana* represented the largest number and was essential for

fruit set. He further reported that pollinating insects greatly increased fruit set in four varieties of apple (Red-, Golden-, Red-delicious, and American mother), pear, plum, and cranberry. Persimmon, peach, and cherry gave a commercial set even in the absence of pollinating insects but the set was higher on flowers receiving insect visits. Singh and Mishra (1986) observed the populations of bees and flies at different elevations in Himachal Pradesh (India), and flies were found to outnumber *A. cerana indica* at all locations on all fruit blossoms. Mishra et al. (1976) reported that visits by honeybees on apple during 1200 and 1500 h were more than all other pollinators and red-delicious had higher fruit set near the bee colonies but that fruit set decreased with distance from the colonies. Verma and Dutta (1986) also compared the foraging behavior of *A. cerana indica* and *A. mellifera* in pollinating the flowers of apple. Without giving the relative abundance of different visitors, Bhalla et al. (1983b) reported that plum, peach, and almond were visited by honeybees *A. cerana indica* along with other insects. Partap (2000a, b, c) summarized the impact of *A. cerana* pollination on the yield and quality of fruit set in peach by 21.9 % over control and 9.4 % over open pollination and in plum by 13.0 % over control and 8.6 % over open pollination (Table 13.5). It also significantly increased the weight per peach fruit by 43.8 % over control and 25.6 % over open pollination and the weight per plum fruit by 38.6 % over control and 10.8 % over open pollination. Similarly, it increased the length per peach fruit by 28.9 % over control and 21.6 % over open pollination and the length per plum fruit by 11.4 % over control and 2.1 % over open pollination, and the diameter per peach fruit by 22.5 % over control and 12.1 % over pollen and the diameter per plum fruit by 13.6 % over control and 4.2 % over open pollination. Bee pollination decreased the time required for ripening of fruit by one week in both peach and plum.

Rai and Gupta (1983) also reported the role of honeybees in apple and pear pollination. Joshi et al. (2003) found that *A. cerana* is much more efficient apple pollinator for the sub tropical region than the *A. mellifera*. Rymahesvskii (1956) has reported that this species spends more than 30 s on the flowers of apple; Verma and Dutta (1986) reported this time as 6.65. Singh (1979) reported maximum number of *A. cerana* (5.66) at 1400 hrs on strawberry flowers. The time spent by any bee on any flower depends on many factors including size of flower and nectar present in the flower. Verma and Dutta (1986) reported that *A. mellifera* visits 3.33 flowers of apple per minute. In the present study, maximum foraging rate of *Apis* species was observed between 1200–1300 hrs, the same observations have also been reported by many workers (Dhaliwal and Bhalla 1980; Raj and Rana 1994; Anonymous 1999; Singh et al. 2006).

Strawberry flowers are also visited by honeybees *A. cerana indica* (Singh 1979) and among the insect visitors, their number was highest in a study in strawberry by Singh (1979). Pandey and Yadav (1970) studied pollination in litchi. They reported that 98–99 % of the visitors were Apoidea and *A. cerana* constituted 28 % of the total visitors. Phadke and Naim (1974) and Dhaliwal et al. (1977) also reported that *A. cerana* constituted 15 % of insect visitors to litchi blooms. Honeybees were the most important pollinating agents of phalsa and the plants of seedling origin were reported to be benefited by insect pollination (Parmar 1976). Fruit yield in lime

Table 13.5 Impact of *Apis cerana* pollination on the yield and quality of fruit of peach and plum in the Kathmandu Valley, Nepal. (Partap 2000a, b, c)

Parameter	Control	Open-pollinated	Bee-pollinated	Increase over control (%)	Increase over open-pollinated (%)	Values of <i>F</i> and <i>df</i>
<i>Peach</i>						
Fruit set (%)	18.04 ± 4.7	30.5 ± 4.3	39.9 ± 3.9	21.9	9.4	<i>F</i> = 11.9; <i>df</i> = 33
Weight per fruit (g)	111.7 ± 16.4	12.7 ± 27.2	160.5 ± 21.7	43.8	25.6	<i>F</i> = 61.81; <i>df</i> = 76
Length of fruit (cm)	8.3 ± 0.8	8.8 ± 0.7	10.7 ± 0.9	28.9	21.6	<i>F</i> = 11.61; <i>df</i> = 77
Diameter of fruit (cm)	18.2 ± 2.5	19.9 ± 1.5	22.3 ± 1.1	22.5	12.1	<i>F</i> = 22.74; <i>df</i> = 77
<i>Plum</i>						
Fruit set (%)	3.9 ± 2.1	8.3 ± 1.9	16.9 ± 1.8	13.0	8.6	<i>F</i> = 18.9; <i>df</i> = 33
Weight per fruit (g)	22.3 ± 5.9	27.9 ± 3.7	30.9 ± 2.9	38.6	10.8	<i>F</i> = 59.65; <i>df</i> = 76
Length of fruit (cm)	4.4 ± 0.5	4.8 ± 0.5	4.9 ± 0.7	11.4	2.1	<i>F</i> = 21.5; <i>df</i> = 83
Diameter of fruit (cm)	11.0 ± 0.6	12.0 ± 0.8	12.5 ± 0.4	13.6	4.2	<i>F</i> = 39.4; <i>df</i> = 71

Values are mean ± S.E.
df degrees of freedom

Table 13.6 Effect of *Apis cerana* pollination on quality and yield of sweet orange. (Partap et al. 2000a, b)

Parameter	Control	Open-pollinated	Bee-pollinated	Increase over control (%)	Increase over open-pollinated (%)
Initial fruit set (%)	15.5 + 5.7	30.7 + 4.9	39.7 + 2.6	24.2	9.0
Fruit drop (%)	50	12.1	4.06	– 45.9	– 2.98
Final fruit set (%)	7.5 + 7.5	26.9 + 6.7	38.09 + 4.4	30.6	11.8
Weight per fruit (g)	146	157.5	170	35.04	7.9
Fruit length (mm)	60.6	64.4	66.0	8.9	2.5
Fruit diameter (mm)	54.9	67.4	74.0	34.8	9.8
Peel thickness (mm)	6.6	5.9	4.4	– 33.3	– 25.2
Juice per fruit (g)	155	180	260	67.7	44.4
Citric acid (%)	2.4	1.7	1.7	– 29.2	No difference
Sugar in juice (%)	9.2	10.8	12.8	39.1	25.5

was found to be increased from 15 to 17 times by the honeybees in Tamil Nadu, India (Anonymous 1981). Coconut was also found to double the fruit yield when honeybee colonies were moved near the trees. On durian (*Durio zibethinus*Murray) in Singapore, honeybees collected pollen and *A. c. indica* visited the plants for nectar (Crane and Walker 1983) but their role in pollination is probably minimal.

In case of sweet orange (Table 13.6), Partap et al. (2000a, b) found that *Apis cerana* enhanced fruit set by 24.2 % and 9 %, and reduced fruit drop by 45.9 and 2.98 % as compared to control and open-pollinated respectively. Bee pollination also enhanced the fruit quality by enhancing the fruit size, fruit weight, amount of fruit juice, and the amount of sugars in the fruit juice. It increased the fruit length by 8.9 and 2.5 % and fruit diameter by 34.8 and 9.8 % as compared to control and open-pollinated respectively. Bee pollination enhanced the amount of fruit juice by 67.7 and 44.4 % and fruit sugar by 39.1 and 25.5 % as compared to control and open-pollinated respectively. It also increased the number of seeds per fruit. Bee pollination reduced the citric acid content by 29.2 % compared to control; there was no difference in the citric acid content in the fruits from bee-pollinated and open-pollinated plants.

Partap (2000a, b, c) studied the effects of *Apis cerana* pollination on the yield and fruit quality of strawberries (Table 13.7) and reported that bee pollination enhanced fruit set by 112.3 and 21.4 % and weight per fruit by 47.9 and 20.3 % in comparison to control and open-pollinated plants respectively. It reduced the percentage of misshapen fruits by 41.7 % and 31.8 % compared to control and open-pollinated plants

Table 13.7 Effects of *Apis cerana* pollination on strawberry fruit. (Partap 2000a, b, c)

Parameter	Control	Open-pollinated	Bee-pollinated	Increase over control (%)	Increase over open-pollinated (%)	Values of <i>F</i> and <i>df</i>
Number of fruits per plant per picking	1.6 + 0.7	28 + 0.9	3.4 + 0.4	112.3	21.4	<i>F</i> = 136.3 <i>df</i> = 87
Weight per fruit	4.8 + 1.9	5.9 + 1.0	7.1 + 0.7	47.9	20.3	<i>F</i> = 85.6 <i>df</i> = 57
Misshapen fruit (%)	64.3	22.5	12.6	–	–	<i>F</i> = 97.1 <i>df</i> = 21

Values are mean + S.E. Statistical results are from analysis of variance
df degrees of freedom

respectively. Abrol and Kumar (2009) also found that the percentage of fruit set was much higher in open-pollinated plants than control (Tables 13.8, 13.9). There was 11.20 % malformed fruit in open-pollinated plots as compared to 17.44 % in controlled one. Insecticides and biopesticides used were found to reduce the pollinator’s population, thereby affecting fruit-yield qualitatively and quantitatively.

The data in Tables 13.8, 13.9 show that there was a considerable improvement in qualitative and quantitative characteristics of strawberry fruit production. There was increase in the fruit set in pollinated crops as compared to those isolated from the insect visits. The percentage of fruit set in open-pollinated and control plots recorded was 70 and 45 %, respectively. There was 11.20 % malformed fruiting in open-pollinated treatment as compared to control (17.44 %). The average weight of the fruit in open-pollinated plants was 15.25 g per fruit as compared to 12.24 g. The volume of the fruit in open-pollinated plot was 16.15 c.c. as compared to control 15.20 c.c. The specific gravity of the fruit in open-pollinated treatment was 0.94 compared to control (0.91). The TSS (°brix) of fruits was 6.83 and 6.72 in open-pollinated and control, respectively. The fruits obtained from open-pollinated crop were less acidic 0.69 % compared to 0.82 % in control.

Abrol (1988) found that *Apis cerana*, *Xylocopa fenestrata*, and *Lasioglossum sp.* were the important flower visitors of almond (*Prunus amygdalus*). Open-pollinated

Table 13.8 Qualitative and quantitative effect of pollination on strawberry attributes. (Abrol and Kumar 2009)

Treatment	Parameters			
	Number of buds	Fruit set	Malformed fruits	Malformed fruits (%)
Open polli-nation	191.66 + 10.40	134.00 + 1.52	15.00 + 1.00	11.20
Control	191.66 + 10.40	86.00 + 1.52	15.00 + 1.00	17.44

Values are mean + S.D. of *n* = 5

Table 13.9 Physico-chemical characters of fruits obtained from different pollination treatments. (Abrol and Kumar 2009)

Treatment	Fruit Parameters							
	Color	Shape	Size	Average weight (g)	Volume	Specific gravity	TSS (°Brix)	Acidity (%)
Open-pollinated	Red	Well formed	Medium	15.25	16.15	0.94	6.83	0.69
No pollination (control)	Partial red	Misshapen	Small	12.24	15.20	0.91	6.72	0.82

TSS total soluble solids

flowers set 30 fruits per 100 flowers as compared to those deprived of insect visits where no fruit set was recorded. Abrol and Bhat (1989) recorded *A. cerana* as important pollinator of Golden Delicious apple flowers. The number of pollen collectors increased from 09.00 h to a peak at 12.00 to 14.00 h and nectar foraging was greatest from 09.00 h to 14.00 h; more bees collected nectar from the side of the flower than from the top, especially early in the day and between 12.00 and 15.00 h. Pollen collectors visited more flowers/min and were more likely to pollinate a flower.

Abrol (1989) recorded *Apis cerana* as the most important visitor to strawberry blossoms and visited an average of 6.2 flowers/min compared with 3.0/min for *Lasiglossum*. Fruit set was 75 % on flowers open to insect pollination, compared with only 47 % on flowers from which insects were excluded. Malformed fruits formed 11 and 23 % of total fruits in open-pollinated and self-pollinated plants, respectively.

Abrol (2005) studied the impact of insect pollination on fruit set in cherry flowers and found that fruit set varied significantly at different distances from the apiary (Table 13.10). Fruit set was significantly higher at 100 m from the apiary (28.20 %) which decreased with the distance and at 500 m fruit set was very low (9.00 %). This clearly reveals that maximum foragers were available at 100 m from the apiary which decreased with the distance, thereby affecting fruit production. Saturation of cherry orchard with different number of bee colonies also significantly affected the percentage fruit set and quantity of fruit production/tree.

He further found that in case of orchards having 5 colonies/ha percentage fruit set and yield were 25 % and 3800 kg/tree as compared to those having 1 colony/ha with 12.2 % and 14.00 kg/trees, respectively (Table 13.11). Evidently, 5 colonies/ha is the most optimum unit for commercial fruit production in cherry.

Table 13.10 Effect of different distances from apiary on fruit production in cherry. (Abrol 2005)

Distance from colonies (m)	Fruit set ^a (%)
0–100	28.20 + 2.28
100–200	24.00 + 2.84
200–300	18.60 + 3.06
300–400	15.80 + 2.92
400–500	9.00 + 1.82

^a Each value is a mean + S.D. of 5 observations

Table 13.11 Impact of number of colonies per hectare on fruit set and fruit production in cherry. (Abrol 2005)

Number of colonies	Fruit set (%)	Yield/tree ^a (Kg)
1	12.20	14.0 + 1.20
2	14.60	17.7 + 1.62
3	17.00	32.3 + 2.12
4	19.50	36.0 + 1.82
5	25.00	38.0 + 1.61

^a Each value is a mean + S.D. of 5 observations

Rahman and Rahman (2000) in Assam, northeastern India, studied the role of honeybees (*Apis cerana indica*) in seed set and yield of buckwheat (*Fagopyrum esculentum*). They found that buckwheat spikes/plant, seeds/spike, percentage of filled seed, and weight of grain were higher in crops pollinated by bees than in open- and self-pollinated crops (Table 13.12). There was a significant increase in crop yield with bee pollination compared with open pollination and the control (without bee pollination).

13.10.2 Vegetable Crops

Several reports are available on the visits of honeybee *A. cerana indica* to cauliflower *Brassica oleracea* L. var. *bottytis* (Rauala 1972; Dhaliwal and Sharma 1973; Adlakha and Dhaliwal 1979; Chakrabarti and Sinha 1980; Kakkar 1980, 1981; Tewari

Table 13.12 Effects of *Apis cerana* pollination on buckwheat yield components (mean values of the years 2000–2002)

Parameters	Control pollination (CP)	Open pollination (OP)	Bee pollination (BP)	Average increase (%)		
				BP c.t. CP	BP c.t. OP	OP c.t. CP
Total number of grains per terminal in florescence	5.32 ± 0.39	27.41 ± 0.58	33.70 ± 0.56	533.46	22.95	415.23
Total number of grains per plant	22.26 ± 1.83	131.36 ± 3.34	169.76 ± 4.10	662.62	29.23	490.12
Fertility (%)	2.49 ± 0.18	13.64 ± 0.27	16.08 ± 0.21	545.78	17.89	447.79
Test weight of 1,000 grains (g)	24.03 ± 0.49	30.14 ± 0.23	33.03 ± 0.26	37.45	9.59	25.43
Grains yield per plant (g)	0.91 ± 0.11	3.77 ± 0.10	4.40 ± 0.12	383.52	16.71	314.29
HI (%)	10.39 ± 0.52	31.50 ± 0.34	35.32 ± 0.35	239.94	12.13	203.18

Values are mean ± S.E. All the mean differences are significant at 0.05 level *c.t.* compared to, *HI* harvest index

and Singh 1983; Verma and Joshi 1983). Thirty-four species of insects, including honeybees, visited the cauliflower blooms at Solan, (Himachal Pradesh), India (Sharma et al. 1974) but *A. cerana indica* was the most abundant (Kakkar 1980) and helped in seed production of this crop (Kakkar 1981). The honeybee was found to prefer cauliflower blooms in comparison to flowers of *Berberis* sp., a weed of the surrounding area (Dhaliwal and Sharma 1973). Adlakha and Dhaliwal (1979) reported that *A. cerana indica* was the most abundant pollinator of cauliflower in Himachal Pradesh and that it was a better pollinator than *A. mellifera*. Rauala (1972) reported that 18 % of the visitors to cauliflower were *A. cerana indica*. That species was also found to be a superior pollinator of cauliflower compared to other visiting insects in northern India (Verma and Joshi 1983). They noted that pod setting, number of seeds per pod, and seed weight were increased by bee pollination. Similar results were reported by Tewari and Singh (1983) and they also reported higher pod set near the hives of *A. cerana indica*. Bhambhure (1957, 1958a) reported the importance of honeybees in fruit yield of cucurbits. In okra *Abelmoschus esculentus* (L.) Moench, bees were the important pollinators (Mishra et al. 1988a) and *A. cerana indica* was the chief pollinator (Chaudhary et al. 1973). Weight and length of capsules of okra and seed number were found to be significantly higher in open-pollinated than in bagged flowers (Mishra et al. 1988b). In onion, *Allium cepa* L., more than 70 % of the total insect pollinators on Patnar-red variety were honeybees and uncaged (insect pollinated) plants produced 72–79 % more seed than caged/bagged plants (Jadhav 1981). Jadhav and Ajri (1981) found 74 % seed set with 3.83 g seed per umbel in open as compared to 8 % and 0.18 g per umbel in bagged inflorescences. Singh and Dharamwal (1970) also obtained 72–79 % higher seed set in bee pollinated crop than in bagged plants. Kumar et al. (1985) found that three onion species, *Allium cepa*, *Allium fistulosum*, and *Allium cepa fistulosum*, were greatly benefited by insect pollination. *A. cerana* was one of the visitors to blooms of these cultivars. Rao and Lazar (1980) observed that *A. cerana indica* constituted 6.83 % of the total visitors to onion blooms and bee pollination increased the seed yield. In bee-pollinated umbels, 93.5 % fruit set took place and each fruit carried an average of 4.3 seeds. The respective figures for bagged umbels were 9.8 % and 1.9 seeds. No reports on *A. cerana* as pollinators of pulses have come to light. However, many pulses are known to set fruit independently of insect pollination (Free 1970).

In case of lettuce (Partap and Verma 1992), the bees started foraging on lettuce soon after flowering head was open, i.e., at 0830 h and ceased their activities only when the flowering heads were closed, i.e., at 1130 h. Peak foraging activity was observed between 0900–1100 h and the average duration of each foraging trip was 15.6 min. Each bee spent an average of 3.84 s on a flower, collected 8 mg of pollen load, and visited 13 flowers per minute. The number of bees per plant was 4. The bees collected only pollen because the plant did not secrete nectar. Bee pollination significantly increased the number of seeds per capitulum (flowering head) by 31.8 and 21.05 % compared to control and open-pollinated plants respectively. Seed weight increased by 16.0 % due to bee-pollination, compared to control, and by 9.2 % compared to open-pollinated plants. Seed length increased

Table 13.13 Qualitative and quantitative effects of *Apis cerana* pollination on lettuce seeds. (Partap and Verma 1992)

Parameter	Control	Open-pollinated	Bee-pollinated	Increase compared to control (%)	Increase compared to open pollination (%)
Number of seeds	15.70 ± 0.63	17.10 ± 0.39	20.70 ± 0.75	31.80**	21.05**
100 seed weight (mg)	109.57 ± 0.46	110.41 ± 0.39	127.14 ± 0.85	16.03**	9.22**
Length of seed (mm)	3.35 ± 0.10	3.73 ± 0.09	4.15 ± 0.10	21.88**	11.26*
Germination (%)	76.66	83.83	96.66	20.00	12.83
Resistance to fungal attack	Susceptible; 6 out of 25 seeds were attacked by fungus	Lees resistant; 2 out of 25 were attacked by fungus	More resistant; no seeds were attacked by fungus		

Values are mean ± S.E.

* $p = 0.05$; ** $p = 0.01$

by 23.8 and 11.2 % and breadth by 13.2 and 3.2 % respectively over that of control and open-pollinated plants. Germination of seeds was also enhanced by 20 and 12.8 % in bee-pollinated plants compared to control and open-pollinated plants respectively. Seeds from bee-pollinated plants also showed resistance to fungal attack (Table 13.13).

Many vegetable crops are completely or partially self-incompatible and incapable of pollinating themselves, so the cross-pollination of their flowers by insects, especially honeybees, is very essential (Verma 1990). Cauliflowers, cabbages, radishes, and lettuces are some of the important vegetable crops grown in the Kathmandu valley of Nepal. Verma and Partap (1994) carried out detailed investigations on the utilization of the native Asian hive bee, *Apis cerana*, to enhance the yield and quality of seed production in these crops through bee pollination. They reported that for both cauliflower and cabbage differences in fruit set, number of seeds per fruit, 100 seed weight, and percent germination between control, open, and bee-pollinated were significant ($p < 0.01$). Bee pollination increased the number of seeds per fruit by 31.8 and 21.05 % and seed weight by 16.03 and 9.92 %, respectively compared to control and open-pollinated plants respectively. The length of seed increased by 23.88 and 11.26 mm and seed germination by 20.0 and 12.83 % compared to control and open-pollinated plants respectively. Seeds from bee-pollinated plants showed resistance to fungal attack.

Verma and Partap (1994) found that bee pollination significantly increased fruit set by 57 and 20 % compared to control and open-pollinated Cauliflower plants

Table 13.14 Qualitative and quantitative aspects of *Apis cerana* pollination on cauliflower and cabbage. (Verma and Partap 1994)

Parameters	Control	Open-pollinated	Bee-pollinated	Increase over control (%)	Increase open-pollinated (%)
<i>Cauliflower</i>					
Fruit set (%)	22.7 ± 1.2	57.0 ± 5.1	80.7 ± 7.5	58.0	23.7
Number of seeds/silique	3.3 ± 1.4	14.8 ± 0.6	19.8 ± 0.6	50.0	33.8
100 seed weight (mg)	264.0 ± 7.0	313.0 ± 8.0	430 ± 12.0	62.9	37.4
Germination (%)	79.6 ± 1.2	83.8 ± 2.0	95.6 ± 1.1	16.0	11.8
<i>Cabbage</i>					
Fruit set (%)	No fruit set	52.1 ± 3.5	79.8 ± 3.3	–	27.7
Number of seeds/silique	–	18.0 ± 0.1	28.3 ± 0.7	–	52.9
100 seed weight (mg)	–	278.0 ± 4.0	420.0 ± 3.0	–	51.1
Germination (%)	–	56.4 ± 1.3	84.0 ± 1.1	–	27.6

Values are mean ± S.E.

Table 13.15 Quantitative and qualitative effects of *Apis cerana* on seed yield of radish seeds

Parameter	Control	Open-pollinated	Bee-pollinated	Increase (%)
Pod set (%)	No pod set	51.2 ± 4.9	74.2 ± 4.4	23.0
Number of seeds per pod	–	5.2 ± 0.4	7.4 ± 0.3	42.3
100 seed weight (mg)	–	1276 ± 4	1844 ± 7	44.5
Germination of seeds (%)	–	44 ± 3.4	76 ± 3.6	32.0

Values are mean ± S.E. Differences in pods set, number of seeds per pod, 100 seed weight (mg), and percent germination of seeds between bee-pollinated and open-pollinated are significant

(Table 13.14). Similarly, the number of seeds per silique increased by 85 and 25 % compared to control and open-pollinated plants respectively. *Apis cerana* pollination also resulted in an increase in the seed weight (by 38.6 and 27.2 %) and enhanced seed germination (by 16 and 12 % compared to control and open-pollinated plants respectively (Table 13.14)). Similarly, bee pollination resulted in an increase in fruit and seed set by 27 and 32 % respectively compared to open-pollinated plants. Control plants did not set any fruit indicating that crop was self-incompatible. Bee pollination significantly increased the weight of seeds by 33.8 % and germination by 28 % compared to open-pollinated plants.

In case of radish, Verma and Partap (1994) reported that *Apis cerana* worker bees started foraging at 0840 h and ceased activity at 1800 h: thus the total duration of foraging was 9.5 h per day. They further found that bee pollination significantly enhanced fruit set by 23 % compared to open-pollinated plants in radish (Table 13.15). Bee pollination also significantly increased the number of seeds per silique (by

Table 13.16 Effect of honeybees on seed setting percentage and seed yield of hybrid sunflower, NDSH1. (Rajasri et al. 2012)

Treatments	Seed set (%)			Seed yield (q/ha)			Increase in yield over control (%)
	2006–07	2007–08	Mean	2006–07	2007–08	Mean	
Open pollination	23.88	10.23	16.52	4.03	8.70	6.4	178
Covered with net	11.88	1.16	6.33	2.06	2.50	2.3	–
Caged with 4 frame colony	19.87	47.1	33.26	3.34	10.08	6.7	191
Caged with 8 frame colony	51.6	50.18	50.89	8.64	11.15	9.9	330
Bee + hand pollination	87.8	60.46	74.13	15.3	13.54	14.4	526
F-test	Sig	Sig		Sig	Sig		
CD	4.90	3.27		0.71	2.29		
CV	6.20	4.6		5.59	17.64		

28.6 %) and seed weight (by 30.8 %) compared to open-pollinated plants. Control branches did not set any fruit, indicating that the crop was self-incompatible. Bee pollination enhanced seed germination by 32 % (Table 13.14).

Partap et al. (2000b) at International Center For Integrated Mountain Development (ICIMOD), Nepal, studied the comparative Foraging Behavior of *Apis cerana* and *Apis mellifera* on Indian Mustard (*Brassica juncea*, var Khumal Broad Leaf) revealed that *Apis cerana* began foraging earlier in the morning (mean time 0626 h) than *Apis mellifera* (mean time 0649 h). In the evening, *Apis mellifera* stopped earlier (mean time 1811 h) than *Apis cerana* (mean time 1821 h). The average duration of foraging activity was 11.55 h for *Apis cerana* and 11.22 h for *Apis mellifera*. Differences in all three parameters were significant at $p < 0.01$. The duration of an individual foraging trip by *Apis cerana* was 23.24 ± 0.22 min., significantly shorter ($p < 0.01$) than the time of 25.29 ± 0.57 min. for *Apis mellifera* (Table 13.15). Both species of honeybee did not differ significantly in behavioral characteristics such as time spent while foraging on each flower and time taken to shift from one flower to another, number of flowers visited on each plant at a time (Table 13.15). Nectar collectors outnumbered pollen collectors ($p < 0.01$) for both species throughout the day, except at 1200 h in *Apis mellifera* when pollen collectors were significantly more than nectar collectors. The ratio of nectar collectors to pollen collectors varied considerably with the time of day and between species at different times of the day (Table 13.16). For *Apis cerana*, more bees were collecting nectar than pollen at 1500 and 1200 h; whereas for *Apis mellifera*, nectar collectors were more numerous at 0900 and 1500 h. pollen foragers of *Apis mellifera* outnumbered those of *Apis cerana* at 0900, 1200, and 1500 h. The peak of activity for *Apis cerana* (mean number of incoming bees/three minutes) occurred



Fig. 13.1 Showing *Apis cerana* working on flowers of *Brassica* spp. and pollination under controlled condition in cages and colonies of honeybees for pollination

between 1200 and 1300 h when the temperature was 25.8–27.4 °C, relative humidity ranged between 52.2 and 58.4, and for *Apis mellifera*, it occurred between 1300 and 1400 h when the mean outside temperature was 25.6–27.4 °C and the relative humidity ranged between 52.2 and 56.6 %.

13.10.3 Oilseed Crops

Apis cerana is known to be an important pollinator of many oilseed crops, especially rapeseed and mustard *Brassica* spp. (Rahman 1940; Latif et al. 1960; Bisht et al. 1980; Bhalla et al. 1983a), niger *Guizotia abyssinica* (Bhambhure 1958b), sesame *Sesamum indicum* L. (Rao et al. 1981), and sunflower *Helianthus annuus* L. (Rangarajan et al. 1974; Thakar 1974; Deodikar et al. 1976; Panchabhavi and Devaiah 1977; Panchabhavi et al. 1976; Wakhle et al. 1978; Basavanna 1979; Bhattacharya et al. 1982; Swaminathan and Bhardwaj 1982). Relative abundance of *Apis* spp. visitors to mustard bloom are given by Naim and Phadke (1976). *A. cerana* had a high degree of fidelity but *A. dorsata* was absent. The flowers of rape *Brassica campestris* var. Pusa Kalyani were well visited by *A. cerana indica* along with other honeybees (Fig. 13.1). The flowers visited by *Apis* spp. had higher pod set, greater

number of seeds per pod, and the weight of seeds was also higher in comparison to those where no pollinators visited (Bisht et al. 1980). *A. cerana* was also found to be an important pollinator of *B. campestris* L. (Mohammad 1940; Rahman 1940; Latif et al. 1960). In niger (*Guizotia abyssinica* (L.f.) Cass.), flowers caged with *A. cerana* produced 2.6 times more seed than in the open, and provision of honeybees in the field was found to increase crop yield. *A. cerana indica* was the most frequent visitor to *Sesamum indicum* (Rao et al. 1981) and was particularly active in the morning, foraging for nectar and pollen. *A. cerana indica* on sunflower showed its highest activity during the afternoon (Rangarajan et al. 1974; Deodikar et al. 1976; Panchabhavi and Devaiah 1977). Seed set in sunflower covered with nylon net was markedly lower than that of an open-pollinated crop (Dhoble and Shinde 1982) as expected for self-incompatible varieties. Moving honeybee colonies to sunflower fields increased seed setting by 27 % (Panchabhavi et al. 1976; Basavanna 1979), and much higher seed yields and weights from plots with *A. cerana* colonies than from plots without bees were reported (Thakar 1974; Deodikar et al. (1976)). Wakhle et al. (1978) reported increases in oil contents after bee pollination in sunflower. Shrivastava and Shrivastava (1986) found *A. cerana indica* and *Xylocopa* sp. as the most important pollinators of sunflower throughout the flowering span of the crop. In pollinated plants, there were 656 grains per head, out of which 226 grains were full. In nonpollinated flowers, none of the grains was full. Bhattacharya et al. (1982) also reported that honeybees were the chief pollinators of sunflower, and excluding honeybees by bagging of floral heads resulted in a reduction in seed yield, seed weight, seed viability, and oil contents.

Rajasri et al. (2012) in a 2-year study on sunflower hybrid NDSH1 found that the crop covered with insect proof net without honeybees recorded significantly lower seed setting % in both the years of study with a mean of 6.33 % compared to other methods (Table 13.16).

All other pollination methods where honeybees were introduced were found to be significantly superior to open pollination (16.52 %). Highest mean seed setting percent of 74.13 % was recorded with bee pollination combined with hand pollination followed by bee pollination with eight frames and four frames (50.89 and 33.26 % respectively). Honeybees + hand pollination gave significantly higher seed yield (14.4 q/ha) than open pollination (6.4 q/ha) and with crop caged with net (2.3 q/ha), thereby the yield was increased about 526 % with honeybee + hand pollination and 178 % with open pollination compared to control plots. There were about 330 and 191 % increased yield of sunflower due to introduction of honeybees of 8 frames and 4 frames colonies respectively. The supplemental honeybee pollination + hand pollination significantly increased the percentage of seed setting and seed yield compared with open pollination and crop in cages without honeybees. The hybrid sunflower seeds obtained from honeybee pollination coupled with hand pollination showed significant superiority with higher percentage of germination accounting for 99 % followed by open pollination (96 %) and bee pollination (95 %) compared to control plot without bees (93 %).

Singh and Singh (1992) studied the impact of self (SP), open (OP), hand (HP), bee (BP), and auto-pollination (AP) on yield and carbohydrate and lipid composition of

Table 13.17 Quantitative and qualitative effects of *Apis cerana himalaya* pollination on rape seed. (Singh et al. 2000)

Yield parameters	Control (PWI)	Open-pollinated (OP)	Bee-pollinated (BP)	CD at 5 %	Increase over PWI (%)	Increase over OP (%)
Silique set (%)	20.67(26.39)	68.42(56.01)	70.23(57.24)	(5.00)	239.77	2.65
Silique length (cm)	3.94(2.11)	4.16(2.12)	4.52(2.24)	(0.07)	14.72	8.65
Number of seeds per silique	9.12(3.14)	11.65(3.52)	13.73(3.80)	(0.24)	50.55	17.85
Weight of 1,000 seeds (g)	2.01(1.66)	2.31(1.75)	2.36(1.76)	NS	17.41	2.16
Seed yield (q/ha)	1.96	12.37	13.45	2.90	586.22	8.73
Seed germination (%)	55.00(47.93)	81.00(65.98)	83.00(66.02)	(5.21)	5.90	2.50
Oil content (%)	33.25(35.18)	38.75(38.48)	40.33(39.41)	(0.81)	21.30	4.10

Figures in parentheses are transformed values
PWI pollination without insects

seeds of *Brassica campestris* L. var. *taria*. The pollination percentage as confirmed by pod formation was 8.1 % (SP), 82.3 % (OP), 88.3 % (HP), and 96.4 % (BP). BP plants were found to produce 3 times heavier pods, 4 times more seeds per pod, 50 times more seeds per plant, 11 times more pods per plant, and 84 times more seed yield per plant than SP plants. Carbohydrate content was inversely proportional to lipid content. In the seeds of SP plants, the total carbohydrate content was about twice that of seeds of BP plants. Triglycerides constituted the majority of neutral lipids. In the seeds of BP plants, triglycerides constituted about 74 % of the total nonpolar lipids, which was about 20 times more than in SP plants. Sterol was the least abundant of all the lipids and phosphatidylcholine was absent from all seeds. High lipid content was directly related to high seed yield, and concentration of total lipid increased according to type of pollination in the order SP OP HP BP. In a similar study, Singh et al. (2000) found that there was significant improvement in qualitative and quantitative parameters of rape when pollinated by *Apis cerana himalaya* (Table 13.17).

From the seed yield data (Table 13.17), it is revealed that % pod setting was significantly higher in plants which had access to insect pollination (95.21 %) than those which were net caged (70.27 %) or muslin bagged (58.81 %). Number of seeds per pod was also significantly higher in open-pollinated flowers (912.62 seeds/pod) in comparison to nylon-caged (5.55 seeds/pod) and muslin-bagged (3.05 seeds/pod). Pods obtained from open-pollinated flowers had significantly more healthy seeds (84.78 %) than those from net-caged (41.45 %) or muslin-bagged (48.37 %) flowers;

Table 13.18 Yield in self pollinated and bee-pollinated crops. (Deodikar and Suryanaryana 1972)

Crop Botanical name	Reported range of seed increase from bee-pollinated over self-pollinated	
	Percent more	Times more
<i>Brassica napus</i> L. (rape)	12.8–139.3	1.128–2.39
<i>Brassica campestris</i> L. var. toria (Toria)	66.0–220.9	1.66–3.20
<i>Brassica campestris</i> L. var. dichotoma (sarson)	222	3.22
<i>Brassica juncea</i> Czern & Coss. (Rai, Indian mustard)	18.4	1.184
<i>Brassica alba</i> Boiss. (white mustard)	128.1–151.8	2.28–2.51

the latter two, however, did not differ from each other. The average weight of 100 healthy dry seeds from muslin-bagged flowers was significantly higher (542.20 mg) than the weight of seeds from open-pollinated (478.50 mg) and net-caged flowers (459.40 mg). Percent oil content of healthy seeds from muslin-bagged (41.64 %) flowers was significantly higher than that from open-pollinated (39.02 %) flowers. However, oil content of seeds from muslin-bagged flowers was equal to that from net-caged flowers. When all the yield parameters were taken into consideration for calculating oil content (mg/pod), it was found that increase in total oil yield from open-pollinated and net-caged plants was 9.76 and 1.55 times more, respectively, than from muslin-bagged plants. Deodikar and Suryanarayana (1972) found enhanced yield in oilseed crops when pollinated by bees (Table 13.18).

In a similar study, Prasad et al. (1989) found that pollination of *Brassica juncea* by *A. cerana* resulted in more siliqua setting, increased length of siliqua, seed weight increased yield and had a pronounced effect on oil contents and germination (Table 13.19).

Mishra et al. (1988b) found that in *Brassica campestris* percent pod setting, number of pods per plant, and proportion of healthy seeds was significantly higher in

Table 13.19 Effect of different pollination treatments on oil and protein contents and germination of seeds in rai cv Pusa bold in Pusa, Bihar. (Prasad et al. 1989)

Treatment	Oil content $\pm$ SE ^a (%)	Protein content $\pm$ SE ^b (%)	Germination $\pm$ SE ^b (%)
Caged with bee	31.80 (34.03 $\pm$ 0.11)	17.80 (4.29 $\pm$ 0.00)	93.30 (9.64 $\pm$ 0.02)
Uncaged	32.10 (34.51 $\pm$ 0.11)	18.44 (4.29 $\pm$ 0.01)	98.00 (9.64 $\pm$ 0.02)
Caged	26.30 (30.85 $\pm$ 0.13)	6.39 (4.05 $\pm$ 0.01)	73.00 (8.54 $\pm$ 0.03)
CV (%)	(0.9)	(0.6)	(0.1)
SE (m)	(3.09)	(0.01)	(0.0)
LSD (5 %)	(0.2)	(0.02)	(0.01)

Average of seven replications

^a Figures in parenthesis are values transformed into angles

^b Figures in parenthesis are values transformed in square root

Table 13.20 Average value of moisture, oil, and protein contents in sunflower seeds variety EC 68414. (Wakhle et al. 1978)

Type of treatment	Number of samples	Moisture	Oil ^a	Proteins ^a	Oil and proteins ^a
SP	6	8.43(0.142)	32.44(2.187)	17.83(2.48)	49.72(3.94)
OP	7	10.197(1.66)	33.978(4.034)	25.55(3.13)	55.47(4.33)
BP	9	9.75(2.048)	38.81(3.41)	17.90(2.952)	56.71(4.35)
CP	12	8.533(0.0681)	38.94(3.045)	20.33(4.051)	58.29(4.62)

Values in brackets indicate standard deviation

^a The figures are percentages of oil and protein contents on moisture free basis

open-pollinated flowers than in net-caged and muslin-bagged ones. Similarly, average weight of seeds and oil content was higher in open-pollinated flowers. *Apis cerana* was the most common pollinating species. The other pollinators observed included *A. mellifera*, syrphid flies, etc. In a similar study, Prasad et al. (1989) found that pollination of *Brassica juncea* by *A. cerana* resulted in more silique setting, increased length of silique, seed weight increased yield, and had a pronounced effect on oil contents and germination (Table 13.19).

Khan and Choudhary (1991) revealed that self pollinated sarson crop in Pakistan produced 34.34 g seed per plant while honeybee (*Apis cerana*) and othetr insect pollinated crop yielded 90.02 g seed and 50 g seeds per plant, respectively.

Wakhle et al. (1978) on the basis of chemical analysis of sunflower seeds obtained from different pollination treatment showed that there was a significant increase in oil (6.5 %) as also oil and protein contents together (7 %) in seeds resulting from bee pollination when compared with self-pollinated seeds (Table 13.20). Evidently, bee pollination is beneficial for not only quantitative but also qualitative improvement in sunflower seeds. In a similar study, Singh et al. (2000) reported that seeds in BP and CP treatments have higher oil percentages and higher oil and protein contents compared to those in SP or even OP treatments. Seeds under OP, BP, and CP treatments showed nearly 6, 7, and 8.5 % increase in oil and protein contents over SP treatment, respectively (Table 13.21).

Panchabhavi et al. (1977) in Karnatka kept 67 *Apis cerana* colonies in a sunflower field of 20 ha and observed that there was 72 % seed set whereas in two other fields which were open to natural pollinators but without colonies there was 55 and 57 % seed set. Kumari and Pandey (2007) found that there was 85.99 % seed setting in the open-pollinated heads followed by 38.15 % (in muslin cloth) and 35.54 % (in butter paper) bagged condition (Table 13.22).

Safflower is basically self-pollinated but bees or other insects are generally necessary for optimum fertilization and maximum yield. Absence of pollinators results in self-pollination. Each branch in the moderately branched stem is terminated by a head which gives rather complex synflorescences, the basic unit of which is the dichasm. The cymes are arranged in nearly corymbose fashion. In this behavior safflower differs from other Asteraceae members which are mostly self-incompatible (Kumari and Pandey 2005). Amongst pollinating insects, Hymenoptera (bees), Lepidoptera (butterflies), and Diptera (flies) play a major role in pollinating the safflower crop. Foraging behavior of bees observed in present study is basically similar to

Table 13.21 Quantitative and qualitative effects of *Apis cerana himalaya* pollination on sunflower. (Singh et al. 2000)

Yield parameters	Control (PWI)	Open-pollinated (OP)	Bee-pollinated (BP)	CD at 5 %	Increase over PWI (%)	Increase over OP (%)
Seed set (%)	2.93(9.84)	60.50(51.13)	71.58(57.81)	(2.20)	2343.00	18.31
Weight of 1,000 seeds (g)	50.32	64.29	54.88	3.26	9.06	17.15
Number of seeds per gram	20.87(4.63)	16.58(4.13)	17.04(4.19)	2.77(0.37)		
Seed yield (q/ha)	2.06	16.60	18.55	1.47	800.49	11.75
Seed germination (%)	69.88(56.78)	86.50(68.80)	91.13(74.55)	(11.94)	30.41	5.35
Oil content (%)	35.96(36.85)	46.79(43.16)	43.70(41.38)	(1.32)	21.52	7.07

Figures in parentheses are transformed values
PWI pollination without insects

Table 13.22 Self incompatibility test in different experimental conditions. (Kumari and Pandey 2007)

Types of pollination	Total number of flowers	Number of filled seeds	Number of unfilled seeds	Seed setting (%)
Naturally pollinated heads	54.75 ± 7.70	47.08 ± 13.78	7.67 ± 6.07	85.99
Butter paper-bagged head	54.76 ± 5.50	19.46 ± 8.88	35.3 ± 3.38	35.54
Muslin cloth-bagged heads	51.53 ± 5.35	19.66 ± 4.67	31.87 ± 0.68	38.15

sunflower (Linsley 1978; Parrish and Bazzaz 1978; Prasad and Subba Rao 1984), in dahlia (Heslop-Harrison and Shivanna 1977) and niger (Panda et al. 1995; Pyke 1984; Reddy 1976; Dhakal and Pandey 2003; Pandey and Dhakal 2004; Neff and Simpson 1990), and in safflower (Kumari and Pandey 2005)

Diwan and Salvi (1965) stated that *Apis cerana* generally ignored peanut flowers, but Heide (1923) stated that the flowers were visited “actively and persistently” by *A. cerana*, and that *A. cerana* visited the flowers from 7 to 9 pm. Gibbons and Tattersfield (1969) reported that *A. mellifera adansonii*, *Nomia* spp., and *Megachile* spp. visited the flowers in the Malawi area of Africa. Leuck and Hammons (1969) stated, “We conservatively estimate that in 1964, at least 80 % of the peanut flowers

were actually tripped for pollen each day by species of the combined bee complex.” Unfortunately, they gave no indication of the bee population density, floral visitation, or bees per unit of flowers that provided this tripping. Hammons et al. (1963) noted that the halictids and megachilids were most abundant during the cool morning hours when most efficient pollination of peanuts occurs, whereas honeybee activity was spread over the day. No consideration was given to changing the degree of honeybee visitation by concentrating their numbers in the area. If the 6–11 % increase, which Girardeau and Leuck (1967) attributed to bee pollination, can be consistently obtained, it is of sufficient importance that consideration should be given to building up the bee population of large peanut plantings. This could be done by “saturation pollination” with honeybees if their use could be proven practical.

It was recorded at Ludhiana that 41 species belonging to 23 families and 7 orders visited *G. hirsutum* and many of them either did not visit floral nectarines or did not touch the sexual parts. It was observed that each flower was visited by 47 insects and *A. dorsata*, *A. cerana*, *A. florea* were the important pollinators; the bees visited 6.7, 6.6, 5.8 flowers per minute and 141, 128, 120 flowers per visit respectively. Only experimental colonies of *A. cerana* were available (Sidhu and Singh 1961). On *Gossypium arboreum*, *A. cerana*, *A. dorsata*, and *A. mellifera* formed 32 % of insect visitors and solitary bees were 27 %; the three honeybees visited 104–124, 94–102, 87–99 flowers per trip, respectively. The plants caged with honeybees (*A. cerana* or *A. mellifera*) gave 10 % more bolls and 15 % more yield and 17 % more seed as compared with those caged without bees. It was observed that *A. cerana* did not touch the anthers but brushed part of the stigma in 60 % of the visits whereas *A. mellifera* touched anthers and brushed past stigma in 70 % of the visits (Tanda 1984, 1985; Tanda and Goyal 1979a, b), indicating the possibilities of cross pollination. *A. mellifera* was present with beekeepers and *A. cerana* came only from experimental colonies. The paradoxical situation in cotton is that beekeeping is not recommended in *G. hirsutum* areas whereas *Gossypium arboreum* can benefit from pollination by *A. mellifera* honeybees. A perfect understanding is needed between the farmers who spray their crop and the beekeepers in that area. The same applies to hybrid cotton.

Clearly most of the oilseed crops are cross pollinated and exclusively depend upon bees and other pollinators for pollination services. In summary, seed yield and yield parameters in different oilseed crops as influenced by bee pollination are given in Table 13.23.

Pollination by honeybees increased the number of fruits per plant and fruits per raceme of *Jatropha curcas* (Atmowidi et al. 2008). Based on measuring the fruit produced by plants, pollination effectiveness of *A. mellifera* was higher than *A. cerana*. The number of fruits per raceme of plants pollinated by *A. cerana* was lowest (2.04 fruits) compared to plants pollinated by *A. mellifera* (3.05 fruits), open plants (2.45 fruits), and control plants (2.29 fruits) (Table 13.24). Pollination by bees increased the number of fruits per plant and fruits per raceme of *J. curcas*. Mean number of seeds per fruit of plants pollinated by *A. cerana*, *A. mellifera*, open plants, and control plants were 2.76, 2.61, 2.47, and 2.63 seeds, respectively. The number of seeds per fruit ranged from 1 to 3 seeds. Results showed that *A. mellifera* was the superior pollinator compared to *A. cerana*. In addition, the number of fruits per plant and fruits

Table 13.23 Seed yield and yield parameters in different oilseed crops as influenced by bee pollination

Crops (variety)	Yield attributes	Pollination	
		With bees	Without bees
Mustard (var. M-27)	Pod set (%)	71.90	48.60
	Seeds/pod	10.80	5.90
	1,000 seeds weight (g)	5.00	2.00
	Seed yield (q/ha)	13.90	1.20
	Oil content (%)	36.40	32.60
Niger (var. M-15)	Seed set (%)	45.80	28.40
	Seed yield (q/ha)	3.79	3.11
	Total florets	45.75	33.42
	Oil content (%)	35.20	32.00
	Average seed set (%)	23.80	14.18
Sesame (var. Kalika)	Seed weight (g)/head	0.80	0.07
	Average yield (q/ha)	8.47	5.64
	Oil content (%)	36.80	36.40
Sunflower (var. modern)	Seed yield (g)/head	23.80	11.60
	Number of seeds/head	540.80	418.25
	Filled seed/head (%)	62.40	58.65
	Seed yield (q/ha)	27.54	9.78

per raceme of plants pollinated by *A. mellifera* were higher than that pollinated by *A. cerana*. Verma (1995) stated that some characteristics of *A. mellifera* were better than *A. cerana*, i.e., duration of foraging trip was significantly longer and worker bees carried significantly heavier pollen loads throughout the day.

Devkota et al. studied the impact of honeybees on seed production of broccoli under lower subtropical terai agro-climatic conditions in Chitwan valley of Nepal and found that both *A. cerana* and *A. mellifera* significantly increased the percent pollination (siliqua set). *A. cerana* pollination contributed in 480.11 and 24.21 % increase in seed set per siliqua over control and natural pollination, whereas the percent increase in the seeds set per siliqua caused by *A. mellifera* pollination were 479.32 and 24.15 over control and natural pollination, respectively. The highest seed yield was obtained from *A. mellifera* pollination (425.880 g/plot) followed by *A. cerana*

Table 13.24 Reproductive success of *Jatropha curcas* pollinated by *A. cerana*, *A. mellifera*, open plants, and control plants. (Atmowidi et al. 2008)

Plant reproductive success	Numbers (+ S.D.)			Control plants
	<i>A. cerana</i>	<i>A. mellifera</i>	Open plants	
Number of fruits per plant	17a (+ 7.94)	19a (+ 11.93)	16a (+ 12.15)	5a (+ 5.13)
Number of fruits per raceme	2.04a (+ 1.65)	3.05a (+ 1.75)	2.45a (+ 1.47)	2.29a (+ 1.25)
Number of seeds per fruit	2.76a (+ 0.51)	2.61a (+ 0.50)	2.47a (+ 0.63)	2.63a (+ 0.52)
Seed weight (g)	0.47ac (+ 0.17)	0.39b (+ 0.14)	0.51a (+ 0.16)	0.41cb (+ 0.11)

Different lowercase letters in the same row indicate different values based on analysis of variance (Anova) and Scheffe test at 95 % level ($p < 0.05$)

Table 13.25 Pollination requirement of different crops (number of colonies per hectare). (Partap 1999a, b)

Crop	Blooming period of the crop	Number of <i>A. mellifera</i> colonies per hectare	Number of <i>A. cerana</i> colonies per hectare	Time of placement of colonies (% bloom)
Mustard and rape	December–January February–March	3–5	5–8	10–15
Niger	August–September	3–5	6–8	5–10
Safflower	March–April	5	4–6	5–10
Sunflower	June	5	8–10	5–10
Coconut	Throughout the year	2–3	4–6	5–10
Sesamum	April–may	2–3	4–6	5–10
Cotton	December–January	3–5	5–8	10–15

(417.500 g/plot) and naturally pollinated (332.75 g/plot). The control plot resulted the lowest seed yield (only 13.35 g/plot). Pollination by both bee species in caged plots increased the pod (siliqua) length significantly over control, but not over naturally pollinated plots. *A. cerana* (3.750 g) and *A. mellifera* (3.637 g) pollinated seeds had significantly higher seed weight (1000 seed weight) over naturally pollinated seeds (3.207 g). This study demonstrated that bee pollination could significantly improve in both the yield and the quality of seeds in broccoli in Chitwan conditions.

13.10.4 Number of Colonies Required for Pollination

Several investigators have attempted to determine the number of colonies of honeybees required for increased yields in rape and their recommendations vary from place to place and crop (Tables 13.25 and 13.26). For instance, Hammer (1963, 1966) recommended three colonies/ha; Radchenko (1964), two; Downey and Bolton (1961), one; White (1970), two; and Vesely (1962), three to four colonies/ha. Although hoverflies appear to play some role in pollination of rape, we consider the honeybee the more efficient pollinator. The ideal pollinator population and proper distribution of colonies for most efficient pollination needs to be determined for various oil crops.

13.10.5 Condiment/Spice Crops

A. cerana is known to be an excellent pollinator of some condiment and spice crops. Narayanan and Sharma (1960) reported that honeybees (*A. cerana*, *A. florea*, and *A. dorsata*) were the chief pollinators of fennel (*Foeniculum vulgare* L.) but the number of *A. cerana* visitors was much lower than that of *A. florea*. Shelar and Suryanarayana (1981) found that seed weight of umbels visited by honeybees was greater than those obtained from caged umbels of coriander. In cardamom (*Elettaria cardamomum* (L.) Maton), the main pollinators appear to be honeybees, particularly *A. cerana* which collects pollen from the flowers in the morning and nectar later.

Table 13.26 Summary of pollination management of different crops. (Partap 1999a, b)

Crop	Blooming period of the crop	Number of <i>A. mellifera</i> colonies per hectare	Number of <i>A. cerana</i> colonies per hectare	Time of placement of colonies (% bloom)
<i>Fruit crops</i>				
Almond	Mid-February to mid-March	5–8	10–12	5–10
Apple	April (7–10 days)	5–8	10–12	5
Apricot	Mid-February (2–3 weeks)	2–3	4–6	5–10
Avocado	April–May	5–8	10–12	10–15
Cherry	February (7–10 days)	2–3	4–6	5
Citrus	March–April	2–3	4–5	5–10
Kiwifruit	March–April	8–9	16–20	5–10
Litchi	March–April	2–3	4–6	5–10
Mango	February	2–3	4–6	5–10
Peach	February–March (3–4 weeks)	1–2	2–3	5–10
Persimmon	March–April (2 weeks)	2–3	4–6	5–10
Plum	February (1–2 weeks)	2–3	4–6	5
Strawberry	February–April (2 months)	> 15	25	5–10
<i>Vegetable crops</i>				
Cabbage	February–March	5	8–10	10–15
Carrot	March–April	5–8	10–12	10–15
Cauliflower	March–April	5	8–10	10–15
Cucumber	June–September	1 for monoecious, 8 for dioecious	2–3 for monoecious, 12–16 for dioecious	10–15
Cucurbits (pumpkin, squash gourd)	June–September	5–8	10–12	10–15
Okra	June–September	1–2	2–3	10–15
Onion	April	5–8	10–12	5–10
Radish	March–April	2–3	4–6	10–15
Turnip	February–March	2–3	4–6	5–10
<i>Oilseed crops</i>				
Mustard and rape	December–JanuaryFebruary–March	3–5	5–8	10–15
Niger	August–September	3–5	6–8	5–10
Safflower	March–April	5	4–6	5–10
Sunflower	June	5	8–10	5–10
<i>Spice crops</i>				
Cardamom	March–April	2–3 ^a	4–6	10–15
Chilli	July–September	2–3 ^a	4–6	10–15
Coriander	February–April	2–3 ^a	4–6	10–15

^a No specific recommendation

The flowers pollinated by bees gave 66 % fruit set, but it was only 11 % in the control flowers (Pattanshetti and Prasad 1973). The plots supplied with bee hives gave 34–35 % higher yield than control (Madhusoodanan and Dandin 1981). Chandran et al. (1980) also found that *A. cerana indica* was the main pollinator of cardamom. Increase of 37.2 and 27.9 % in fruit set was observed in Malabar and Mangirabad varieties of cardamom respectively in plants having access to bee visits compared to plants which had no insect visits (Chandran et al. 1980). Siddappaji and Channabasavanna (1980) reported that honeybees constituted 98 % of visitors to cardamom flowers and even one bee visit was sufficient to pollinate the flower. The cardamom panicles exposed from a layer of leaf mulch to open pollination by bees improved fruit set by about 14 times (Pattanshetti and Prasad 1974).

13.10.6 Fiber Crops

A. cerana indica is found to visit some fiber crops also, e.g., cotton *Gossypium herbaceum* L. (Sidhu and Singh 1961, 1962; Tanda and Goyal 1979a, b), congo jute *Urena lobata* L. (Crane and Walker 1983), tussa jute *Corchorus olitorius* L., white jute *C. capsularis* L. (Kundu et al. 1959) and sunnhemp *Crotalaria juncea* L. (Jitendra Mohan 1973). On Asiatic cotton, 6 % of the total visitors were from *A. cerana indica* and their role in pollination of cotton flowers was established (Tanda and Goyal 1979a, b). Sidhu and Singh (1961, 1962) studied the insect of cotton and found that plants caged with *A. cerana indica* and *A. florea* gave 17.19 % more seed cotton than plants without insects. Sidhu and Singh (1962) studied the insect of cotton and found that plants caged with *A. cerana indica* played no role in pollination, perhaps due to the larger size of the flowers. The bees robbed the nectar by simply biting through the lateral part of the keel (Jitendra Mohan 1973). Honeybees have also been reported collecting nectar from the flowers of congo jute in Indonesia (Crane and Walker 1983) but no information on its pollination is available. However, Kundu et al. (1959) reported that in tussa jute and white jute, the rate of cross pollination was 12 and 2.3 % respectively, and only small numbers of bees visited the flowers.

13.10.7 Forage/Fodder Crops

A. cerana is known as a visitor to the flowers of some forage or fodder crops, e.g., alfalfa (*Medicago sativa* L.) (Shelar and Suryanarayana 1983), berseem *Trifolium alexandrinum* L. (Narayanan et al. 1961), fenugreek *Trigonella foenum-graecum* L. (Crane and Walker 1983), and horse gram *Macrotyloma uniflorum* (Lam.) Verdc. var. *uniflorum*. Shelar and Suryanarayana (1983) recommended 12.25 colonies of *A. cerana* per hectare for the pollination of alfalfa (*M. sativa*). The berseem seed set per head is about 70 %, provided that enough pollinating insects, which are essential for profitable seed production, are present. *A. cerana* has been reported to pollinate

the flowers of berseem in India (Narayanan et al. 1961) but wind was found to affect adversely the activity of the bees (Dhaliwal and Atwal 1976). *A. cerana* has also been observed collecting nectar from the flowers of fenugreek in Maharashtra, India (Crane and Walker 1983) but their role in pollination of this crop has not been reported. Plots of horse gram pollinated by *A. cerana* gave yields 6 times higher than plots caged to exclude insects (Anonymous 1981).

13.10.8 Crops Producing Dyes

The only two dye-producing crops where *A. cerana* has been reported as pollinator are Java indigo (*Indigofera arrecta* A. rich.) and Sumatran indigo (*Indigofera tinctoria* L. var. *tinctoria*). The flowers set seed only if they are tripped by bees (Howard and Howard 1915). When they were tripped artificially (resulting in self-pollination only) less seed was set than when they were visited by bees.

Number of colonies required for different crops are given in Table 13.26.

13.11 Value of *Apis cerana* Pollination in Mountain Areas

Mountain areas are characterized by inaccessibility, fragility, marginality, diversity, niche, and adaptation mechanisms (Jodha 1990). *Apis cerana* beekeeping system fits well with these characteristics and supports the livelihood of mountain people. Several mountain areas are inaccessible, lack transport and communication infrastructure, so in these circumstances migratory beekeeping with *Apis mellifera* becomes highly expensive, vulnerable, and high-risk business. Stationary beekeeping system with *Apis cerana* is more suitable and fits well in mountain farming systems and processes. Mountains are fragile and their productivity is hampered by uncertain rainfall, low fertility, and lack of agriculture inputs. Cycle of negative changes keeps on hitting the mountains, which results a nonconductive situation for *Apis mellifera* beekeeping, as it requires more ideal conditions for economic returns. On the other hand, *Apis cerana* keeps on going under these adverse conditions; even if everything goes wrong, colonies abscond and farmer does not lose anything as the bees reoccupy their hives when conditions allow them to do so.

Pollinators also play an often unrecognized role in combating soil degradation by enhancing the replenishment cycle, i.e., more pollination, more seed, more plants, returning more biomass to the soil, more food for birds, insects, and other animals. Globally, the annual contribution of pollinators to the agricultural crops has been estimated at about 153 billion Euros. For the Hindukush–Himalayan region it is estimated that the contribution of pollinators to the agricultural economy of the selected areas of the HKH region including Chittagong Hill Tracts of Bangladesh, Bhutan, Himalayan Hengduan mountain areas of China, northwestern Indian Himalayan states of Himachal Pradesh and Uttarakhand, and northern Pakistan is 2.69 billion US Dollars per year (Table 13.27). The figure could be double if Afghanistan, Northeastern Indian States, Myanmar, and Nepal were also included in the study.

Table 13.27 Economic value (in million US\$) of insect pollinators (EVIP) to different crops in the selected study areas of the Hindu Kush-Himalayan region. (Partap et al. 2011)

Study areas	Crops						
	Fruit crops	Oilseed crops	Pulses	Spices crops	Tree nut crops	Vegetable crops	All crops
Chittagong Hill Tracts, Bangladesh	33.09	0.97	0 ^a	0.09	3.39	14.27	51.81
Bhutan	10.81	0.82	0.31	0.67	0.96	3.99	17.56
Chinese Himalayas	453.09	188.05	0 ^a	4.03	0 ^a	27.64	672.81
Kashmir	407.66	–	–	–	9.73	8.22	425.61
Himachal Pradesh, India	353.02	1.28	1.58	0.02	0.05	7.84	363.79
Uttarakhand, India	159.43	3.85	0.79	0.57	0 ^a	2.04	166.69
Nepal	54.38	23.82	1.78	0.25	0.26	0.19	80.76
Mountain areas of Pakistan	879.75	38.08	0 ^a	–	36.44	0 ^b	954.27
All study areas	2,183.48	273.8	36.98	4.26	28.33	126.60	2,685.3

^a Crops/varieties grown are entirely self-pollinated

^b Producer price data were not available

13.12 Favorable Pollinating Characteristics of *Apis cerana*

In addition to its general pollinating ability, *A. cerana* possesses several positive characteristics which make it a superior pollinator, even compared to *A. mellifera*. Such characteristics include foraging behavior, foraging rate, foraging range, flower constancy, and colony strength (Jhajj and Goyal 1979a, b). *A. cerana* foragers show the usual foraging behaviors on crops as is known for *A. mellifera*, i.e., the foragers include only pollen gathers, pollen as well as nectar gatherers, and only nectar gatherers. The different proportions of these categories of foragers vary during the course of the day and flowering span of the crop (unpublished).

A. cerana has a higher wing-beat frequency (305 ± 16.2 per second) than does *A. mellifera* (235.2 ± 7.5) (Goyal and Atwal 1977) and its foraging rate is also higher on *B. juncea* (unpublished). These characteristics should make it a more efficient pollinator, at least in certain categories of crops where smaller number of pollen grains are required for pollination and flower size is small, e.g., cruciferous, umbelliferous, and many papilionaceous crops.

The small foraging range of *A. cerana* (about 1 km) in comparison to *A. mellifera* (3–4 km) (Goyal 1978; Punchihewa et al. 1985) may be an additional advantage to breeders and seed growers. This is because the foragers would tend to restrict themselves to smaller radii where pesticidal operations could be regulated more effectively by the growers and, if desired, the breeders could establish the isolation yards for genetic purity of a cultivar easily by keeping *A. cerana* as a pollinator

for self-pollination. This would be especially beneficial in cruciferous crops where contamination of genotypes is common (Adlakha and Dhaliwal 1979).

A. cerana has been reported to have a high floral fidelity (Chaudhary 1978) and out of 5,600 pollen loads analyzed, only 56 contained pollen from more than one plant species. Dhaliwal and Atwal (1986) reported that only 66.5 % of *A. mellifera* foraging on alfalfa carried pure pollen.

A. cerana colonies have smaller strength (not greater than 30,000) than those of *A. mellifera*, which can reach even 60–70,000 (Mishra and Sihag 1987). The larger size of *A. mellifera* colonies presents problems for management of pollination and smaller nuclei or packages are often used. This problem is overcome if *A. cerana* is used as a pollinator of crops. The colonies with smaller strength and small honey stores can be transported with little difficulty. However, for large crop fields, higher number of colonies of *A. cerana* would be required. *A. cerana* is also better adapted to higher altitudes as compared to *A. mellifera*.

Thus, it is clear that *A. cerana* is a pollinator of a large number of cultivated crops. Like *A. mellifera*, *A. cerana* can also be easily managed. In spite of it being less of a producer than *A. mellifera*, several of the favorable pollination attributes of *A. cerana* put this bee ahead in terms of its usefulness as a pollinator of crops not only possibly in areas of its natural distribution but also in new ones where it is not indigenous.

13.13 Special Attributes of *Apis cerana*

Apis cerana plays a dominant role in developing countries of Southeast Asia in boosting the productivity of agricultural crops and improving the quality of fruits and seeds through pollination activities. The studies have revealed that in comparison to honeybee *Apis mellifera*, this native bee species (*Apis cerana*) offers several comparative advantages as a pollinator of agricultural crops. Studies have shown that *Apis cerana* offers several comparative advantages over *Apis mellifera* as pollinator. These include initiation of early foraging at lower temperatures, longer foraging hours, shorter flight range, no competition for food and nesting sites with other bee species, coevolution with native crops, more suitable for glass house pollination, better searching ability for sparse floral resources. Moreover this bee species is more docile and industrious in nature, less prone to attacks of wasps, and a high level of resistance to nosema disease and parasitic mites. *Apis cerana* can coexist with other native bee species and require little chemical treatment of colonies to control epidemics. However, as yet, this native bee species has not become popular among beekeepers because of several behavioral characteristics. These include their frequent swarming and absconding, their tendency to rob, their production of a large number of laying workers, and their lower honey yields. These negative traits show ecogeographical variations depending upon the subspecies/geoecotypes and management efficiency of the beekeepers and are amenable through basic and action research.

13.14 Advantages of *Apis cerana* Over *A. mellifera* as a Crop Pollinator

Based on the above observations on foraging behavior and also data obtained by other investigators it could be argued that *A. cerana* may be preferred over *A. mellifera* for the pollination of agricultural crops. *A. cerana* begins foraging (pollination) activities at a lower temperature (3–5 °C cooler) than *A. mellifera*. Further, peak foraging activity of *A. cerana* occurs at temperature 5.6 °C cooler than *A. mellifera*. Thus *A. cerana* could be used for crops blooming in early spring and at latitudes at least as far north (or south) where *Apis mellifera* is used. The flight range of *A. cerana* is less than half of *A. mellifera*. This point is of particular interest from point of view of pollination of small plots of specific crops. An *Apis cerana* hive can be placed in the vicinity of a specific crop as it would restrict to pollinating that crop and does not wander or escape.

The average daily duration of foraging activity of *A. cerana* is longer than that of *A. mellifera*. This is because of their early initiation and late cessation of foraging activity. *Apis cerana* may thus ensure adequate pollination of the crop in bloom in less period of time, particularly during adverse weather conditions (monsoon or frosty spring seasons) when honeybee activity becomes severely limited. As a result of loss of habitat due to severe deforestation, and rapid agricultural transformation, both food sources (nectar and pollen) as well as nesting sites are increasingly becoming a limiting factor throughout the range of *A. cerana*. Introduction of the exotic honeybee, *A. mellifera*, will accentuate these limitations because it would compete for both the food and physical (resting space) resources. This will eventually lead to reduction in population of native pollinators or maintain them at much lower levels than if this exotic honeybee species were not present.

The deleterious effects of such competition are more particularly likely between *A. mellifera* and *A. cerana* as both of them occupy the same ecological niche and are also more closely related to each other in habits, constitution, and structure. Further, since *A. mellifera* is more aggressive and prolific than *A. cerana* according to competitive exclusion principle of Hardin (1960), it could be expected that this exotic honeybee species will completely displace *A. cerana*. A large number of fruit, vegetable, oilseed, and other crops have evolved in association with *A. cerana* or other native pollinators. It is likely that these plants have developed symbiotic relationship with the pollinators.

Exotic *A. mellifera* may disturb such coexistence and inefficiently pollinate native crop plants' coexistence and reduce their reproductive success. Beekeeping with *A. mellifera* in comparison to *A. cerana* requires high cost technology which the small and marginal farmers in developing countries of Asia cannot afford. *A. mellifera* also requires chemical treatment of colonies to control epidemics which is undesirable both from economic and environmental health point of view.

Beekeeping with *A. cerana* is a centuries old traditional occupation which forms an integral part of cultural and social heritage of rural communities of Asia. Such an environment-friendly occupation/craft may die through the introduction of exotic *A. mellifera*.

13.14.1 Apis cerana: A Potential Crop Pollinator for Global Commercial Development

Apis cerana could potentially be of great importance to beekeeping and pollination events outside its range. New technologies in molecular biology will present opportunities to introduce genes that code for desirable characteristics of *A. cerana* into population of *A. mellifera* and vice versa.

However, introduction of *A. cerana* outside its range for global commercial development would require following testing and monitoring:

1. Identification of commercially valuable subspecies/geographic ecotypes of *A. cerana* in its native habitat;
2. Selection and testing of the stock on offshore islands;
3. Zonation of areas for *A. cerana* and *A. mellifera* beekeeping. Such zonation would solve the problem of interspecies competition.

13.15 Pollination Management

13.15.1 Problems Associated with Bee Pollination

The second half of the twentieth century saw the widespread introduction of organic insecticides, compounds that were initially developed during the Second World War. Little is known as to how much effect these compounds have on wild bees in natural situations. Pesticide risk assessments are routinely carried out for honeybees, but the results from these are probably not directly applicable to bumblebees because they have different floral preferences, and are active at different times of the day (Thompson and Hunt 1999). For example, pyrethroids are commonly applied to flowering oilseed rape in the early morning or evening, to avoid honeybees. Pyrethroids are repellent to most insects, so that sprayed crops are avoided by honeybees. However, spraying in the early morning or evening is likely to result in direct contact with foraging bumblebees since these are precisely the times when bumblebees are most active. This problem is exacerbated by the higher toxicity of pyrethroids at low temperatures (Inglesfield 1989). Stimulated by the growing use of bumblebees in glasshouses for crop pollination, laboratory and field bioassays appropriate to bumblebees have been developed (van der Steen 1994, 2001), but these are not widely used; therefore, a few toxicological data are available (Thompson 2001). There are three possible routes of exposure for bumblebees to agrochemicals; through direct contact with sprays (such as when sprays are applied to flowering crops or drift onto flowering weeds where bees are foraging); through contact with contaminated foliage; and through uptake of chemicals in nectar. The latter is most likely with systemic insecticides. Tests with dimethoate and carbofuran suggest that they are selectively transported into the nectar, where they can reach high concentrations (Davis and Shuel 1988).

Given the large volume of nectar consumed by bumblebees, this could prove to be the most important route of exposure.

Despite risk assessments, widespread poisoning of honeybees has been reported in fields of oilseed rape in the UK and elsewhere (Free and Ferguson 1986). In Canada, the use of the insecticide fenitrothion in forests led to a decline in yield of nearby *Vaccinium* crops due to a reduction in abundance of bumblebee pollinators (Ernst et al. 1989). In the UK, bumblebee deaths have been reported following applications of dimethoate and of alphacypermethrin to flowering oilseed rape, and of lambda cyhalothrin to field beans (Thompson and Hunt 1999; Thompson 2001). Most insecticides are broadly toxic against both honeybees and bumblebees (Thompson and Hunt 1999), and their inappropriate use will inevitably lead to bee mortality.

However, aphid control becomes a problem during pollination when the crop is in bloom and bees are actively foraging in the field (Abrol 1997, 1993; Abrol and Andotra 1998, 2003). Insecticides presently registered for use in seed crops are considered highly toxic to bees. Thus, to avoid killing bees, pollination often must be interrupted. The colonies are either temporarily removed from the field, or applications must be made at night. In either case, several days can pass before the bees resume normal foraging activity. Repeated applications to control aphid populations may cause a significant disruption in pollination and reduced seed yield and quality.

Flowering *Brassic*as and other oil crops attract not only a large number of insect pollinators especially the honeybees for nectar and pollen but also other insects which feed on flowers, leaves, fruits, and thereby causing serious economic losses (Sihag 1991; Abrol 2007, 2008, 2009). Oilseed crops are attacked by aphids, caterpillars and bugs during flowering and pod formation stage. This requires the application of insecticides to combat the pest (Sihag 1986, 1988, 1991; Sihag et al. 1999a, b) which poses serious problem for the foraging activity of honeybees and developing brood. Evidently, problems of infestation of aphids and other pests and their management during flowering period of rapeseed and mustard crops poses major problem for their utilization as effective pollinators for crop production which also get incidentally killed. Aphid control becomes a major problem during pollination when the crop is in bloom and bees are actively foraging in the field. Insecticides presently registered for use in seed crops are considered highly toxic to bees. Thus, to avoid killing bees, pollination often must be interrupted. The colonies are either temporarily removed from the field, or applications must be made at night. In either case, several days can pass before the bees resume normal foraging activity. Repeated applications to control aphid populations may cause a significant disruption in pollination and reduced seed yield and quality.

Pollinator–plant interaction is a very complex phenomenon and is influenced by many overlapping effects. The protection of pollinators, including honeybees, is as essential as the protection of crops from the insect-pest damage. The use of pesticides for pest control on the one hand and the role of honeybees (*Apis* spp.) for crop pollination on the other have become essential components of modern agriculture. Unfortunately, these two practices are not always compatible, as honeybees are susceptible to many of commonly used pesticides (Johansen 1977; Russell et al.

1998; Cunningham et al; 2002, Sundararaju 2003) used for the control of insect pests (Poehling 1989; Stark et al. 1995). The major constraint confronting pollinator–plant interaction is the indiscriminate and excessive use of pesticides for controlling insect-pests (Bisht et al. 1980; Rana and Goyal 1991; Zhong et al. 2004). The loss of honeybees directly affect beekeeping through loss of honey production and indirectly the crop production due to inadequate pollination. Reduction of population of these beneficial insects due to insecticides, therefore, incurs significant environmental, ecological, and economic costs (Bai and Reddy 1977; Pimentel et al. 1980; Crane and Walker 1983; Prakash and Kumaraswami 1984; Khan and Dethle 2004).

Honeybees constitute a major group of insect pollinators and their pollinating efficacy is manifested not only through increase in yield but also by the improvement of the crop quality through heterosis breeding (Melnichenko 1976). The bees earn about 10 million Rupees to the national exchequer in terms of honey production and beeswax, and it is expected that an additional crop yield worth 90 million Rupees could be obtained due to pollination of crops (Sorthia and Chari 1985). Levin (1984) estimated that the value of crops in the United States of America that benefited directly from the honeybee pollination approaches US\$20 billion annually. The annual cost of crop loss due to insecticides poisoning of pollinating honeybees has been estimated at US\$135 million in the United States of America (Pimental et al. 1980). Notwithstanding with the absence of such estimates, the indispensability for a harmonious compromise between pest management and honeybee pollination of crops in India cannot be gainsaid.

Bee poisoning or killing of bees from pesticides continues to be a serious problem for beekeepers. Most bee kill occurs when pesticides are applied or allowed to drift on to blooming crops or weeds (Mayer 2003). Most (99 %) bee kills result from bees picking up the pesticides when foraging. The hazards of insecticidal application on flowering crops include—direct mortality, fumigative effects, repellent effect, and toxicity of residues present on various floral parts and in nectar to the insect visitor. A highly toxic insecticide generally reduces the field force of a colony within a short period of time. Colonies may be reduced by one third to half in strength within 24–48 hours (Eckert and Shaw 1960), thus adversely affecting both the production and marketing segments of the honey and beekeeping industry. Generally, fumigative action of insecticides used under field conditions is of much shorter durations than the effect of contact and stomach poison. A prolong repellent effect will deprive the flowers of pollination benefits of insect visits, while a short repellency will deter the insect pollinators from visiting the treated bloom for a brief period and thereafter, allow them to resume their foraging activity (with minimum residual hazards) without compromising with the yield potential of the crop.

Poisoning of insecticides to honeybees is generally more pronounced because of their long hours of working on the crop flowers for pollen and nectar collection, continuous working nature, and long flights with pollen loads. Bee poisoning is more chronic in areas with lack of sufficient wild pollens and nectar plants to sustain the number of colonies required for crop pollination. Most bees are pastured in agricultural areas where they are subjected to killing from pesticides (Mayer and

Johansen 1983). The beekeeper has little or no control of when and what pesticides are applied in the areas of his bee forage (Mayer 2003).

Furthermore, modern agricultural practices have resulted in the reduction of wild insect pollinators and disturbed insect–flower relationship by way of disappearance of wastelands and uncultivated strips of land, destruction of certain food sources by weed control and overall changes in the environment. Wild bees are also damaged by pesticides. Poisoning may result from contaminated food as well as from florets, leaves, soil, or other material used by the bees in nesting. The toxicity of a specific insecticide to honeybees and wild bees is not always the same, and even among wild bees some materials are more toxic to one species than to another.

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13.15.2 Pesticide Application Practices to Reduce Bee Poisoning

1. Apply pesticides only when needed.
2. Choose the pesticide with the lowest hazard rating for bees, particularly the lowest residual toxic effect, from the list of pesticides available for a particular pest control program.
3. Liquid or granule applications are less hazardous than dusts. Microencapsulated forms of pesticides have a significantly longer residual life than other application forms. The minute capsules can be carried back to the colony in the same manner that pollen is carried, and can kill brood and young adult bees.
4. Ground application is less hazardous than aerial application, particularly when applied in close distances to apiaries. Fine sprays are less toxic than coarse sprays.
5. Where practicable, apply pesticides when bees are not active on the crop. Because pesticides are considered a low hazard when they have dried, application in the early morning may be suitable. For pesticides with a residual toxic effect of a few hours, apply in the late afternoon or early evening.
6. The time of day when a pesticide is applied should be chosen to minimize the risk of spray drift occurring either over apiaries or over plants being foraged by bees. Treatments can and should be applied only when bees are not foraging for nectar or pollen. If bee-attractive plants need to be treated while in bloom, they should be treated at night or in the early morning or late evening when the bees are not flying.
7. Where practical, beekeepers should be given prior notice, preferably a minimum of 48 hours, of a pesticide application to allow apiaries to be moved from the area.

13.15.3 Pollination Recommendations

Honeybees are the most effective agents involved in cross pollination of rapeseed, mustard, and other oil crops. In modern agriculture, farm mechanization and high yielding varieties (HYV) are very common. In such a condition, to increase the yield more inputs such as water, fertiliser and other agro chemicals are in use. Indiscriminate use of pesticides/fungicides often kills a large number of pollinators. In certain cases, a single crop over a vast area is cultivated. This also reduces number of wild honeybee colonies in those areas. Therefore, the importance of bee keeping in the field is being realized as an important input to increase the production of oilseed crops.

1. It is the only pollinating insect which can be employed in large numbers and distributed at desired places.
2. Honeybees have a flower fidelity to one kind of a plant at a given time than either solitary bees or bumblebees.
3. It works for longer periods than most of the solitary bees.
4. Its body parts are specially modified to pick up large number of pollen grains.
5. It is less affected by adverse weather conditions than most other insect pollinators.

For effective pollination and increased yield of oilseed crops, the efficiency of a bee colony as a pollinator would depend upon the following factors:

13.15.3.1 Colony Strength

Larger and stronger colonies are five times better than the smaller and weaker ones because the former have a higher percentage of older bees as foragers. Thus, good honey yielding colonies are better pollinators also.

13.15.3.2 Number and Time of Placement of Colonies

This factor depends upon density of plant stand, total number of flowers in inflorescence of each plant, duration of flowering, strength of bee colonies, number of flowers over an area of 1 ha of land. Generally 3 to 5 colonies of *Apis mellifera* per hectare of crop in bloom and five strong colonies of *Apis cerana* are recommended for sufficient and efficient pollination.

13.15.3.3 Distribution of Colonies in the Field/Orchards

Honeybees primarily visit the nectar flow source which is in the range of 0.3 to 0.5 km radius from the apiary. Beyond 0.5 km range, pollination activity greatly diminishes. For efficient pollination, hives should be placed singly than in groups. Bees tend to forage in the area which is closest to their hive, particularly when weather is not favorable.

13.15.3.4 Time and Placement of Colonies

Generally, colonies are to be introduced when 5 to 10 % of the crop is in bloom. Earlier placements of the bees result in foraging in other weeds and wild plants in the vicinity, but ignore the crop bloom. If bees are moved too late, they can only pollinate late and less vigorous ones.

13.15.3.5 Weather Conditions

Failure and success of bee pollination depends upon the weather conditions as it affects equally the crop as well as bees. *A. cerana* can forage at a lower temperature than *A. mellifera*. Wind velocity of more than 15 miles/h affects the forage behavior, therefore wind breaks is recommended around the orchard/field. Cool, cloudy weather and storms greatly reduce bee flights.

13.16 Pollinator Decline and its Impact on Agricultural Productivity

In recent years, pollinator populations and diversity have been declining worldwide (Partap and Partap 1997, 2002; Partap et al. 2001; Ahmad et al. 2003; Eardley et al. 2006). Evidence of this decline is available in some intensively cultivated areas of the Himalayan region. Studies carried out in some apple farming areas of the Himalayan region, e.g., Maoxian County of China, Himachal Pradesh Province in India, Balochistan Province in Pakistan, Thimphu and Paro valleys in Bhutan and Jumla district in Nepal, revealed that inadequate pollination has severely affected apple production. The yield has gone down and the quality of fruit was inferior due to inadequate pollination caused by the lack of natural pollinators of apple, which forced farmers to manage pollination of their cash-crops (Partap and Partap 2002). Recently, Ahmad et al. (2003) recorded other evidence of pollinator decline in Kaski district in Nepal. They reported a decline in the number of *Apis laboriosa* nests at eight sites from 182 nests in 1986 to 48 in 2002. They found that while the number of nests declined substantially at three sites, four sites were completely deserted by the bees.

Researchers throughout the world are convinced that the factors causing this decline could be the loss of habitat, with the accompanying decrease in food supplies (nectar and pollen) as a result of decline in pristine areas, land use changes, increase in monoculture-dominated agriculture, and the negative impacts of modern agricultural interventions, e.g., use of chemical fertilisers and pesticides (Verma and Partap 1993; Aizen and Fiensinger 1994; Partap and Partap 1997, 2002; Allen-Wardell et al. 1998; Ahmad et al. 2003). Clearing of forests and grasslands for farming has resulted in the loss of nesting sites and food sources of the pollinators. This has been clearly revealed by our studies carried out in mountain areas of China, India, and Pakistan

where apple orchards are being extended into the forestland and the grassland (Partap and Partap 2002). Changes in climate might also be affecting insect numbers (Partap and Partap 2002). Other factors, such as insufficient focus and capacities of national institutions and changing economic and social landscapes, have further aggravated this decline in the populations of indigenous honeybees throughout large parts of the Himalayas.

Increases in monocropping have also reduced the diversity of plants providing food for natural pollinators. Earlier, farmers used to grow a variety of crops, which bloomed during different months of the year and provided food for a number of natural insect pollinators, but the transformation of agriculture from traditional mixed-crop farming to high value cash-crop farming in recent years has led to an increase in monocrop-dominated agriculture. Examples of this are available in several pocket areas of the Himalayan region where farmers have switched over to the cultivation of apples and other fruit crops and off-season vegetable farming (Partap and Partap 2002). Further, modern industrial farming techniques such as use of pesticides and other agrochemicals may also be contributing to a decline in natural pollinators, especially wild bees, which could be playing a much greater role in pollinating crops. Studies carried out by ICIMOD (Partap and Partap 2001, 2002) revealed a serious lack of pollinators in apple farming areas of the Himalayan region due to heavy and indiscriminate use of pesticides on apple and other cash-crops. Similar examples are available from many areas around the world. Claire Kremen and her colleagues (quoted in Washington Post, December 16, 2002, p. 10) reported that farms using large amounts of pesticides were also frequented less often by the bees in Sacramento Valley in California (www.uoguelph.ca/~iucn/ Accessed on April 16, 2003).

Increases in honey hunting practises and ruthless hunting of the nests of wild honeybees are another reason for the decline in population of indigenous honeybees. For example, in Nepal, in the past, honey-hunting formed a part of tradition and a source of income for the communities; at present it is being commercialised and exploited by big contractors and companies (Ahmad et al. 2003). Development of infrastructure and tourism are other contributing factors which may have made adverse impact on populations of wild bees and other pollinators through destruction of the nesting sites and food sources. Introduction of invasive exotic species to an area is yet another factor affecting populations of native species. This may lead to the competition for food, for nesting, and transfer of pests and diseases from one species to another.

The introduction of *Apis mellifera* to increase honey production has led to a decline in beekeeping with indigenous *Apis cerana* in several mountain areas. The reasons were complex: (1) large scale efforts made by all government, nongovernment, and private entrepreneurs to promote this European bee to all areas without realising that this bee may not be suitable to small-scale beekeeping and to remote and high altitude areas; (2) the introduction of *A. mellifera* without providing proper training on management of this species; (3) transfer of pests and diseases between *A. mellifera* and the indigenous species and vice versa; (4) competition for floral resources; and (5) human factors, e.g., temptation of keeping exotic species to get more honey, etc. (Partap and Partap 1997). The occurrence of forest fires also affects

pollinator populations by not only destroying the nesting places and food sources of the pollinators but also killing them. Lastly, changes in climate might also affect insect numbers (Partap and Partap 2001, 2002).

This decline in pollinator populations and diversity presents a serious threat to agricultural production and conservation and maintenance of biodiversity in many parts of the temperate, subtropical, and tropical world. Its impacts include reduced agricultural productivity and biodiversity. One indicator of the decline in natural insect pollinators is decreasing crop yields and quality, despite necessary agronomic inputs. Examples can be found in Himachal Pradesh in northwest India, northern Pakistan, and parts of China where, despite all agronomic inputs, production and quality of fruit crops such as apples, almonds, cherries, and pears is declining (Partap and Partap 2001, 2002; Partap et al. 2001).

Studies further revealed that because of the pollinator decline in parts of China (e.g., Maoxian county), farmers are forced to pollinate their crops, e.g., apple and pears by hand using human pollinators, which is an expensive and time consuming method of pollinating agricultural crops owing to the increased scarcity of labor and costs. Similar stories are also available from other countries, e.g., Pakistan where, disappointed with the very low yield and quality of apples as a result of poor pollination, several farmers in Azad Jammu and Kashmir have chopped out their apple trees and are using this land for other purposes. Thus, there is a pressing need to protect, promote, and diversify pollinator resources in several countries of the developing world. This calls for initiating research and extension activities in this direction and developing strategies to conserve pollinator populations. This will require much more understanding of multiple services provided by pollinator diversity and the factors that influence them, including farmers, in order to secure sustained pollinator services in agricultural ecosystems.

13.17 Management of Honeybees for Crop Pollination

The efficiency and effectiveness of a honeybee colony in pollination of various crops depend upon a number of factors (Verma 1990; Free 1993). The quality of pollination is, therefore, determined by the number of colonies per unit area, strength of bee colonies, amount of brood in the colony, number of foraging bees, condition/health of the colony, placement of colonies in the field, time of placement of bee colonies and the weather conditions. Scientists have developed farmer-friendly techniques for using honeybees for pollination (Kozin 1976; Verma 1990; Free 1993; Gupta et al. 1993; Partap 1999a, b). A simple method of crop pollination is to place honeybee colonies in a field when the crop starts flowering. Experience from pilot experiments has shown that the best results are achieved by placing strong bee colonies (having large amounts of unsealed brood and are free of diseases) at the time of 5–10 % flowering in the crop. However for effective pollination, according to Verma (1990), Free (1993), and Partap (1999a, b), the following things should be kept in mind while using bee colonies for crop pollination.

13.18 Strength of the Colony

Large, strong colonies are better pollinators than small colonies because larger colonies have more forager bees. Also, good honey-yielding colonies are more efficient pollinators. Research has shown that a bee colony with 60,000 worker bees produces one-and-a-half times more honey than four colonies with 15,000 workers each (Verma 1990; Free 1993; Verma and Partap 1993). The same is true for pollination. Thus, as far as possible, strong colonies should be used for pollination. The colonies should be well settled, disease-free, and have young, laying queens. The strength of a honeybee colony depends above all on the availability of bee forage and season. Generally, colony strength is poor during winter because of low temperatures and a dearth of bee flora. Thus, when required in early spring for pollination of temperate fruit crops, these colonies are not strong enough to act effectively. To help colonies maintain their strength, bee colonies should be moved to low hill/plain areas during winter because it is warmer and floral sources are available and returned to the hills in spring when temperate fruit and vegetable crops are blooming. Such migration of bee colonies is the practise in Himachal Pradesh in India, northwest Frontier Province (NWFP) and Punjab in Pakistan, and northern parts of China (Partap and Partap 2002).

13.19 Number of Colonies

The number of colonies required for pollination depends on the total number of plants, total number of flowers per plant, attractiveness of the flowers to bees, duration of flowering, amount of nectar and pollen, bee species used, strength of colonies, number of pollen foragers, and amount of unsealed brood in the colonies (Kozin 1976; Verma 1990; Free 1993). In general, three strong colonies of *A. mellifera* per hectare of crop are recommended for adequate pollination (Kozin 1976; Verma 1990; Free 1993). Colonies of *A. cerana* are smaller: a 10-frame full-strength colony of *A. mellifera* is three times larger than a 10-frame full-strength colony of *A. cerana*. However, the foraging rate of *A. cerana* is 1.5 times greater than *A. mellifera*. Therefore, four to five strong colonies of *A. cerana* per hectare of crop are required. The number of colonies required also varies from crop to crop and from season to season for the same crop. The summary of pollination management of different crops is provided in Table 13.26.

13.20 Time of Placement

Time of placement of bee colonies is important for high yield and good quality produce. It is important to synchronize flower opening and foraging activity of bees. Freshly migrated colonies are more likely to visit a crop than those in place for a

long time. Colonies should be brought to the field when 5–10 % of the crop is in bloom. If colonies are placed early, bees will forage on flowers of wild plants nearby, becoming conditioned to these, and ignoring the target crop when it blooms. If bees are moved late, they will pollinate only late on less vigorous flowers, resulting in poor yields and low-quality produce. For effective pollination of crops that flower for a short period, such as plum, bees should be moved when plants just start blooming because 50 % flowering is achieved within 3–4 days.

13.21 Distribution Colonies in Field

Honeybees prefer to visit sources of nectar and pollen that are near to their colonies (200–300 m). At over 500 m, pollination activity diminishes greatly. For effective pollination, colonies should be placed singly instead of in groups and distributed evenly in the field. Field experience has also shown that the distribution of colonies in an orchard depends upon the pollinators in proportion to the orchard. Gupta et al. (1993) have suggested the placement of varying number of *A. mellifera* colonies on fruit set in apple orchards having different proportions of pollinators. Based on the findings of their experimental research, these authors suggested that in apple orchards with 20 % polliniser proportion, bee colonies should be placed in groups of four, and each group separated by 50 m gives maximum fruit set. However, with those with a higher polliniser proportion, four to five bee colonies should be distributed in the orchard to give a good fruit set.

13.22 Condition of the Colonies

Bees that forage for pollen (pollen collectors) are known to be better pollinators than nectar collectors. Colonies being used for pollination should have large amounts of unsealed brood. This will increase the pollen requirement and the colony will recruit more pollen foragers. The amount of unsealed brood in a colony can be increased by adding frames of unsealed brood from another colony that is not being used for pollination. Pollen collection can also be increased by taking out frames in which the bees have stored pollen.

13.23 Directing Foraging Bees to Target Crops

For crops that are poor nectar producers and relatively unattractive to honeybees, e.g., kiwifruit, bees should be fed sugar syrup in which a few fresh flowers and pollen of the crop have been soaked for some hours. Feeds should be given at night or early morning before bees go foraging. This increases pollen collection the following day and thus pollination.

13.24 Avoiding Competitive Sources

Sometimes bees ignore the crop to be pollinated and forage on other more attractive plants, e.g., weeds nearby. In such cases, removal of weeds to avoid competition in attracting bees is necessary, and the use of good agronomic practises for a healthy crop such as proper manuring, irrigation, and pesticide application is advisable. Usually, however, weeds are useful as they provide additional nectar and pollen sources. Some crops are less attractive to bees or not at all. In such crops, flowers can be sprayed with a honey or sugar solution to attract more foragers.

13.25 Weather Conditions

Weather plays an important role in determining the success of pollination. It affects both the foraging activities of bees and fruit/seed setting by plant. Cool weather and wind affect foraging activities of bees. Therefore, colonies should be placed in sunny, sheltered locations giving protection from wind to encourage maximum flights in spring. Where no natural windbreak is available, provide a temporary wind shelter.

13.26 Payments for the Pollination Services of Honeybees

Examples from Himalayas

As pointed out earlier in this chapter, a wide diversity among species, including agricultural crops, depends on, or is benefited by, honeybee pollination. Honeybees are therefore essential for “food diversity”, biodiversity, and the maintenance of natural resources. It has been argued that the main significance of honeybees and beekeeping is considered in pollination of crops and natural flora whereas hive products are of secondary value. It has been estimated that the benefit of using honeybees for enhancing crop yields through crosspollination is much higher than their role as producers of honey and beeswax. Morse and Calderone (2001) showed that the value of honeybee pollination to crop production in the United States is US\$14.6 billion annually. Similar estimates have been made for other countries. For example, the value of honeybee pollination in Canadian dollars is 1.2 billion per year in Canadian agriculture (Winston and Scott 1984), about US\$150 million in UK (Carreck and Williams 1998), and US\$2.3 billion per year in New Zealand (Matheson and Schrader 1987). Cadoret (1992) estimated that the direct contribution of honeybee pollination to increased farm production in 20 Mediterranean countries was US\$5.2 billion per year, US\$3.2 billion in developing countries, and US\$2 billion in other countries.

Keeping in view the contribution of honeybees to agriculture, honeybees are being used for perfecting pollination, especially in developed countries like the United

States, Canada, Europe, and Japan that have long used honeybees for pollination of crops, such as apples, almonds, pears, plums, cucumbers, melons, watermelons, and berries. However, the developing countries lag far behind in making use of honeybees for crop pollination. Even though plenty of scientific evidence is now available to prove that honeybee pollination increases the productivity of various cash-crops, the practise of using honeybees for crop pollination does not yet exist in these countries.

However, recently, realizing that the fruit industry has been suffering heavily because the service of bees was no longer adequate, local governments, particularly in Himachal Pradesh and Maoxian County (southwestern Sichuan province, China), have been undertaking special efforts to promote managed pollination of apples since late 1990s. While in Himachal, the government promoted honeybees for pollination of apples, the government in Maoxian initially promoted “hand pollination”. In 1990s, apple orchards in Maoxian county of China were pollinated by hand using “human pollinators”. These people were paid 15–20 Yuan per day in addition to providing them food for their pollination services (Partap and Partap 2000, 2002; Partap et al. 2000a, b). Now, even in China the local institutions are also promoting beekeeping for pollination of apples and other crops.

Partap (2011) in her survey in apple farming areas of Bhutan, China, India, Nepal, and Pakistan revealed that farmers in Himachal Pradesh in northwestern Indian Himalayas are using honeybees for apple pollination. Himachal is the only place where honeybees are being promoted for apple pollination. It is interesting to note that while in the United States, the first colonies of honeybees, *A. mellifera*, were rented for pollination of pears in Virginia in 1895 (Waite 1895) and for apple pollination in 1909 in New Jersey (Morse and Calderone 2001), in Himachal Pradesh, the honeybees were first rented for apple pollination only recently in 1996. Use of beekeeping for pollination of cash-crops is of great benefit not only to the farmers but also to the beekeepers. Beekeepers receive money for the pollination services of honeybees, and farmers’ income is increased through boosting crop productivity as a result of pollination services of bees. Every year tens of beekeepers with hundreds of bee colonies move to various hills and valleys of Himachal Pradesh to provide pollination services to apple farmers and get paid in return for their services.

13.27 Promoting Honeybees for Pollination

While much research remains to be done to bring pollination into rural development and land management, the author is of the opinion that there is need for integrating pollination as an input in agriculture and horticulture development packages of practices. This will help promote beekeeping for pollination. However, the challenge is great: in fact, the importance of pollination to agricultural husbandry and biodiversity conservation have not been recognized by policy makers, planners, development workers, and farmers in most countries of Asia. There is no conceptual clarity or recognition of the value of pollination. The main constraints to promoting honeybees for managed pollination are lack of awareness and understanding among

farmers, extension workers, planners, and policy makers about the importance of pollinators and pollination, lack of integrating pollination in agricultural development packages, scarcity of managed colonies of honeybees, and lack of knowledge about conservation, rearing, and use of pollinators and their pollination behavior. Partap (2000a, b, c) and Partap and Partap (2002) discuss the strategies to promote honeybees for pollination. According to them, this would require the following.

13.28 Raising Awareness

Lack of awareness at all levels—be it farmers, extension workers, and professionals at policy and planning level—is one of the main problems in promoting managed pollination. With a few exceptions, farmers in those areas where there is a pollination problem are not aware of the value of honeybees (including other pollinators) for agricultural production. This is both because beekeeping has always been promoted exclusively as an enterprise for honey production and because cash-crop farming is a new activity in many developing countries, and there is no indigenous knowledge of the need for managed crop pollination for enhancing cash-crop production. Raising awareness at all levels about the importance of managed crop pollination through beekeeping and other pollinators is the first step as part of development efforts.

13.29 Pollination As a Technological Input in Agriculture

Pollination has been overlooked in agricultural development strategies and is not included as a technological input in agricultural development packages. High value agriculture is being promoted in several areas, and extension institutions offer packages of practices for each type of crop, but the importance of managing pollination to achieve higher yields has been overlooked. Thus, farmers have no way of knowing how essential it can be. This weakness in the agricultural extension system needs to be addressed. Since pollination is essential for the production of fruit and seeds, it should be included in agricultural development packages by promoting beekeeping for crop pollination as a “double benefit approach”. Thus, the most important step in promoting the wider use of honeybees for crop pollination is to include beekeeping as part of agricultural development efforts. Including managed pollination in agricultural development packages will also help develop strategies to conserve, promote, and use other pollinators.

13.30 Influencing Thinking About Bees and Beekeeping

Traditional thinking is that beekeeping is for honey production; its role in crop pollination is rarely considered. Today, most government agencies are only engaged in promoting beekeeping for honey production. The move toward introduction of *A. mellifera* to increase honey production is an example of this. Thus, there is a

need to change the general mindset about honeybees and beekeeping and to raise awareness about the importance of managed crop pollination.

13.31 Strengthening Research and Development Institutions

Managed crop pollination is a relatively new area. There are few institutions with explicit mandates or expertise for research and extension in this area. Most institutions are working only with beekeeping and promoting it as a cottage industry to increase family income through the sale of honey. Promoting the value of honeybees as reliable pollinators of agricultural crops will require special efforts to strengthen research and extension systems. This is necessary in order to underline applied research in key areas of managed crop pollination. Issues such as decline of pollinator populations and the need to conserve them need to be addressed by the institutions.

13.32 Capacity Building and Human Resources Development

Lack of knowledge among farmers about the pollination behavior of honeybees is another constraint hindering the use of honeybees for crop pollination. Even those farmers, who do know that they can use honeybees to increase apple pollination and yield, do not always know how to use bees. Though linked with the institutional strengthening, it requires more focus to build the capacities of individual farmers, development workers, and farmer-led organizations that are the agents of change. There is need to train farmers and beekeepers in managing honeybees for crop pollination. There is also a need to develop human resources and build their capacities to initiate activities in the areas of conserving, rearing, and using pollinators to improve pollination and thus agricultural productivity.

13.33 Conclusions and Future Strategies

The foregoing discussion reveals that by proper management and supplementing field with honeybee colonies, crop productivity can be enormously increased. The honeybee pollination not only increases yield in self-incompatible varieties but even the self-fertile varieties give many times increased yield over those deprived of insect visits. There is also significant increase in quality of the crop and other parameters through the process of heterosis. Honeybee pollination stimulates germination of pollen on stigma of flowers and improves selectivity in fertilization, increases the viability of embryos and seeds, enhances resistance to diseases and other adverse environmental conditions, increases nectar production in the nectaries of plants, results in early and uniform crop set, and increases oil content in oilseed crops. The

study suggests that both protective applications of pesticides use of bees should be integrated in a manner to boost oilseed production and honey production.

There is strong evidence that pollinators are declining as a result of local and global environmental degradation (Kluser and Peduzzi 2007). Recent declines in both wild and domesticated pollinators, and parallel declines in the plants which rely upon them possess a serious threat to global food security and sustainable agriculture (Potts et al. 2010). Because a sizable proportion of the human diet depends directly or indirectly on animal pollination, the issue of how decreases in pollinator stocks could affect global crop production is of paramount importance. Agriculture has become more pollinator dependent because of a disproportionate increase in the area cultivated with pollinator dependent crops. If the trend toward favoring cultivation of pollinator-dependent crops continues, the need for the service provided by declining pollinators will greatly increase in the near future.

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Chapter 14

Safety of Bees in Relation to Pest Management

14.1 Introduction

The poisoning of bees by pesticides is a major problem affecting the efficiency of bees not only in the production of honey but also in crop pollination. This problem is not limited to any one country but the world as a whole. The problem is complex with many ramifications, frequently interwoven with emotion. The greater part of the problem is associated with insecticides applied to cultivated crops—cotton, fruits, vegetables, grains, and legumes. Damage also results from treatment of forests and rangelands, and even suburban areas, for the control of pests of man and animals.

By nature, honeybees from a colony visit flowers over an area of several square miles. The intensity of visitation in any one part of the area is determined by the relative attractiveness of the flowers. The extent of damage to the colony by a pesticide application is influenced not only by the relative toxicity of the material, the number and methods of application, the time of day, and the weather conditions, but also by the number of bees from the colony visiting the flowers in the treated area, the type of food (nectar or pollen) they are collecting, the type of flowers the food is collected from, the season of the year the damage occurs, and even the influence of forage available to the bees for weeks before and after the application.

Wild bees are also damaged by pesticides. Poisoning may result from contaminated food as well as from florets, leaves, soil, or other material used by the bees in nesting. The toxicity of a specific insecticide to honeybees and wild bees is not always the same, and even among wild bees some materials are more toxic to one species than to another.

Honeybees are required for the pollination of many vegetable and fruit crops. Without adequate populations of bees, the production of these and other crops would be impossible. In a world where people expect and demand more food and fiber each year, all branches of agriculture must continuously adapt and improve to meet this challenge. Farmers now find it essential to annually increase efficiency and production to remain in business and to show a profit. Practically every agricultural crop has insect pests that sometimes require treatment. Unfortunately, beneficial insects such as honeybees are also susceptible to pesticides. The use of pesticides for

pest control on the one hand and the role of honeybees (*Apis* spp.) for crop pollination on the other have become essential components of modern agriculture. Without either of the two, global food production would be seriously impaired. Unfortunately, these two practices are not always compatible, as honeybees are susceptible to many of commonly used pesticides (Johansen 1977; MacKenzie and Winston 1989; Russell et al. 1998; Cuninghame et al. 2002; Sundararaju 2003), used for the control of insect pests (Poehling 1989; Stark et al. 1995). The major constraint confronting pollinator–plant interaction is the indiscriminate and excessive use of pesticides for controlling insect-pests (Bisht et al. 1983; Rana and Goyal 1991; Zhong et al. 2004). The loss of honeybees directly affect beekeeping through loss of honey production and indirectly the crop production due to inadequate pollination. Reduction of population of these beneficial insects due to insecticides, therefore, incurs significant environmental, ecological, and economic costs (Bai and Reddy 1977; Pimental et al. 1980; Crane and Walker 1983; Prakash and Kumaraswami 1984).

In India, 50 million hectare of area is under entomophilous crops, cross pollinated by different abiotic and biotic agents. About 90 % pollination is carried out by insects, 85 % of which comprise the bees (Singh et al. 1989). Honeybees constitute a major group of insect pollinators and their pollinating efficacy is manifested not only through increase in yield but also by the improvement of the crop quality through heterosis breeding (Melnichenko 1976). The bees earn about Rs. 10 million to the national exchequer in terms of honey production and beeswax and it is expected that an additional crop yield worth rupees 90 million could be obtained due to pollination of crops (Sorthia and Chari 1985). Levin (1984) estimated that the value of crops in the USA that benefited directly from the honeybee pollination approaches US\$ 20 billion annually. The annual cost of crop loss due to insecticides poisoning of pollinating honeybees has been estimated at US\$ 135 million in the USA (Pimental et al. 1980). Notwithstanding the absence of such estimates, the indispensability for a harmonious compromise between pest management and honeybee pollination of crops in India cannot be gainsaid.

Bee poisoning or killing of bees from pesticides continues to be a serious problem for beekeepers. Most bee kill occurs when pesticides are applied or allowed to drift on to blooming crops or weeds (Mayer 2003). Most (99 %) bee kills result from bees picking up the pesticides when foraging. The hazards of insecticidal application on flowering crops include—direct mortality, fumigative effects, repellent effect, and toxicity of residues present on various floral parts and in nectar to the insect visitor. A highly toxic insecticide generally reduces the field force of a colony within a short period of time. Colonies may be reduced by one third to half in strength within 24–48 h (Eckert and Shaw 1960), thus adversely affecting both the production and marketing segments of the honey and beekeeping industry. Generally, fumigative action of insecticides used under field conditions is of much shorter durations than the effect of contact and stomach poison. A prolonged repellent effect will deprive the flowers of pollination benefits of insect visits, while a short repellency will deter the insect pollinators from visiting the treated bloom for a brief period and thereafter, allow them to resume their foraging activity (with minimum residual hazards) without compromising with the yield potential of the crop.

Poisoning of insecticides to honeybees is generally more pronounced because of their long hours of working on the crop flowers for pollen and nectar collection, continuous working nature, and long flights with pollen loads. Bee poisoning is more chronic in areas which lack sufficient wild pollens and nectar plants to sustain the number of colonies required for crop pollination. Most bees are pastured in agricultural areas where they are subjected to killing from pesticides (Mayer and Johansen 1983). The beekeeper has little or no control of when and what pesticides are applied in the areas of his bee forage (Caron 2000; Mayer 2003). Conservation of honeybees for crop pollination is vital to agricultural production (Bisht et al. 1983; Sorthia and Chari 1985; Smirle 1990; Gupta 1994; Dhuley et al. 1996; Russel et al. 1998; Kremen et al. 2002).

The long term effects of insecticides might be due to persistent physiological changes or due to delayed exposure to pesticides that have been incorporated into a colony's beeswax or stored in food. The chronic feeding of low amount of pesticides deleteriously affected such important colony characteristics as worker population's size, honey production, and brood rearing (Stoner et al. 1983; Webster and Peng 1989). In addition, pesticides reduced the worker longevity (Smirle et al. 1984), homing behavior (Thompson 2003), temporal division of labor (MacKenzie and Winston 1989), poor defense against wax moth, and inability to remove debris (Nation et al. 1986), impaired the ability to communicate the location food source to other workers (Schricker and Stephen 1970), loss of queen (Stoner et al. 1985a) or disruption of queen rearing, (Nunamaker et al. 1989) and foraging behavior patterns i.e., dance rhythms, flight velocity, walking speed, wing beat frequency, etc. (Waller et al. 1979; Cox and Wilson 1984). Ruijter and Steen (1987), Atkins and Kellum (1986) and Czoppelt and Rembold (1988) observed amorphogenic effects in delayed and abnormal development.

Many colonies that are not killed outright may be weakened to such an extent that they are no longer effective as pollinators or honey producers, nor can they be divided to increase the number of colonies. This type of economic loss far exceeds the loss from colonies being killed outright by pesticides. Though the monetary loss to the beekeeping is large, yet the value of the seed and fruit lost through the lack of pollination is estimated to be 50–100 times greater (Atkins 1975).

Pesticide application may also change the physiology of nectar and pollen producing plants, change the attraction of bees to flowers, affect pollen viability, and reduce pollen germination on contaminated stigma (Eaton 1963; Church and Williams 1978; Fell et al. 1983; Kuriachen et al. 1992; Sharma 1993). All these effects of pesticides usages are serious to pollination potential and honey production.

The immature stages of honeybees are vulnerable to insecticidal poisoning that result in hidden damage to honeybee colony (Davis and Shuel 1985; Naumann and Isman 1996; Davis and Shuel 1988). Loss of brood and new bees as result of exposure of insecticides may be more deleterious than the loss of foragers (Singh and Kumar 1998) because the latter could be replaced more quickly and provide less potential value to the colony than emerging workers. Insecticides poisoning to immature stages of the honeybees is important to beekeepers and agriculturists alike because, replenishment of the adult population is involved. The insecticides interfere

with the normal growth and development of honeybee larvae. Exposure of brood to an insecticide is mediated by adult workers. Entry of an insecticide into brood may occur by oral, dermal, or respiratory routes (Witmann and Engels 1981, Witmann 1982; Davis and Shuel 1988). Insecticides are amorphogenic that is, it affects the development process in such a way that abnormalities are visible in the pupa or adult (Atkins and Kellum 1986). The contaminated pollen and nectar fed to brood may poison and kill larvae in all stages and the effects of the pesticides may be delayed so that death occurs among pre-pupae, pupae, and newly emerged adults.

Insecticides may cause low hatchability of eggs, easily ruptured larvae (Barkers and Taber 1979), abnormalities in post larval stages (Beetsma and Ten 1975; Ruijter and Steen 1987), including induction of queen-like characteristics in worker larvae (Czoppelt and Rembold 1980), premature tanning (Davis et al. 1988), abnormal size (Czoppelt and Rembold 1980; Davis et al. 1988), unsuccessful larval and pupal molts (Rembold et al. 1980), low pupation success (Davis et al. 1988), abnormal hormonal balance in larvae (Beetsma and Ten 1975), and amorphogenesis (Atkins and Kellum 1986) in larvae poisoned by insecticides. Many abnormalities resulting during development from egg to adult may mimic the brood diseases (Abrol 1997b).

The use of insecticides for insect pest control and honeybees for crop pollination have become essential components of modern agriculture. Honeybees (*Apis* spp.) are one of the most essential biotic component, useful to man because of their contribution to high honey and agricultural yield and in maintaining the ecological balance by perpetuating wide varieties of wild plants through pollination. The honeybees visit the flowers for collecting nectar and pollen and thus are vulnerable to direct or indirect contact with the treated surface where pesticides are applied for crop protection (Abrol 1997). The major constraint confronting pollinator–plant interaction is the indiscriminate and excessive use of pesticide for controlling insect pests (Bisht et al. 1983; Zhong et al. 2004). The extensive and diverse literature on the effect of pesticides on honeybees (*Apis* spp.) reflects the complicated nature of the problem as well as its economic significance (Anderson and Atkins 1965; Johansen 1977; Webster and Peng 1989; Sharma and Abrol 2005). Some pesticides persist in the nectar and pollen and in concentrations high enough to cause mortality of foragers and brood. The residues at lower concentration in nectar and pollen are transported to hive (Johansen 1972; George and Rincker 1982; Tasei and Carre 1985; Tasei et al. 1987) and these accumulate in the bodies of developing larvae proving hazardous. The immature stages of the honeybees are vulnerable to insecticidal poisoning, which may result in hidden damage to honeybee colony (Davis and Shuel 1985; Davis and Shuel 1988; Davis et al. 1988). Pesticidal poisoning, therefore, is posing a serious threat to the survival and existence of beekeeping industry (Atkins 1975; Thomas and Phadke 1994; Pandey and Prasad 1995; Khan and Dethe 2004). Different pesticides had been found to exhibit variable toxicity to bees (Anderson and Atkins 1966; Rahman et al. 1997). Italian honeybee, *Apis mellifera* L. and indigenous bee, *Apis cerana indica* F. are effective pollinators and honey producers. Their use in agriculture and horticulture is increasing in the areas where the pesticides are also used for crop production. Pesticides caused invisible damage to bee colonies (Davis and Shuel 1985) and show visible toxicity to honeybees (Dhaliwal and Singh 2000).

Pesticides used against the insect pests are the main poisoning agents to honeybees and other pollinators (Hameed and Singh 1989).

The problem of bee poisoning is as old as about 1870s when Thompson (1881) detected accidentally killed bees by application of Paris green to pear trees in bloom and some sort of bee malady was found around beehives at that time. The intermediary period between 1870 and 1888 contributed further to the knowledge of the toxicity of Paris green and London purple. Brose (1888) demonstrated that these insecticides in sugar syrup repelled the bees to some extent but those which fed on syrup were killed in 1–4 h. Troop (1918), Hoskin and Harrison (1934) reported some inorganic compounds like arsenic highly toxic to honeybees. Likewise, Kingsmill (1917) reported accidental killing of bees to a great extent from Paris green and molasses bait mixture. Bourne (1927) reported that blossom treated with a mixture of lead arsenate, lime sulfur, and nicotine sulphates were unattractive to bees.

The review that follows describes the effects of insecticidal poisoning on foraging honeybees, brood in the laboratory and field tests estimating the acute, sublethal, and chronic toxicity related to *A. mellifera* and *A. cerana indica* F. under different environmental conditions. The available information pertaining to the problem under investigation is reviewed under the following subheads:

14.2 Pesticidal Poisoning to Bees

A lot of information is available on honeybee losses or mortality due to insecticidal poisoning. Shaw and Mendall (1940) conducted extensive survey and found a huge a loss of bees due to indiscriminate use of insecticides. After World War II, rapid development, expansion, and uncontrolled of new organic insecticides as a part of modern agricultural methods has severely threatened the survival of honeybees. The losses of bees resulting from insecticidal poisoning has been very high and are on an increasing trend (Johansen 1969; Stevenson 1971; Morse et al. 1987). Johansen (1977) reported 70,000 colonies of bees destroyed by pesticidal sprays in USA since 1967. Mayer and Johansen (1983) reported low to moderate mortality of honeybees which occurred periodically during 1979 to 1981. Serious losses of honeybees were observed in most of the apiaries in South Korea. Choi and Lee (1986) reported honeybee losses approximately ranging from 10–50 % per year. Pankiw and Jay (1992b) observed that sprayed colonies of *A. mellifera* gained significantly less weight than control colonies and further, the numbers of dead bees were significantly higher in sprayed colonies. Hood (1998) investigated the pesticide application pattern of bee colonies, increase in management cost caused by mites, and the effect of pesticides used by others on bee keeping activities. Of the surveyed bee keepers, agricultural pesticide-related colony losses have been reported to be increased by 15 %, over the past few years.

Celli and Porrini (1991) took efforts to prepare and present the maps on “Risk Areas” for most frequently used crop protection chemicals and discussed the use of honeybees for monitoring environmental pollution. However, most of the available

reports are from western countries and no report on losses of the honeybee colonies due to pesticidal usage is available from India.

14.3 Toxicity of Different Insecticides to Honeybees

In general, commonly used insecticides can be classified into four groups, viz. organochlorine, organophosphates, carbamates, and synthetic pyrethroids. The available literature on toxicity to honeybees of different pesticides is as follows:

14.3.1 Toxicity of Organochlorine Insecticides

Organochlorine compounds are nerve poisons and affect the ion permeability of the nerve receptors (Matsumura 1985). The effect of organochlorine insecticides on honeybee in the laboratory and in the field has been studied extensively (Anderson and Atkins 1958a, b, 1966, 1968; Johansen 1966). In India, the first study was carried out by Cherian and Mahadevan (1946) with dichlorodiphenyltrichloroethane (DDT) and gammaxene against *A. cerana indica*.

Singh (1968, 1969) reported endosulfan to be least toxic to *A. cerana* and had no effect on foraging intensity and was of no harm to bees. Endosulfan was reported to be a simple poison of low toxicity. The 1–2 days old larvae were least susceptible. The LD₅₀ for all larvae was 1.3 times that for adults (Atkins and Kellum 1986).

In laboratory studies, endosulfan and menazon were least toxic to bees (Hameed et al. 1973). Similar results were obtained by Singh et al. (1974) with respect to menazon and endosulfan against honeybee (*A. cerana*) workers with the LC₅₀ values of 0.4826 and 0.4503 %, respectively. Menazon (0.01 %) and endosulfan (0.05 %) might be considered as nontoxic at recommended rates (Attri and Sharma 1969). Bai and Reddy (1977) found organophosphates and carbamates showing similar toxicity which was greater than those of chlorinated hydrocarbons. Kapil and Lamba (1974) evaluated the contact toxicity of 11 insecticides and reported endosulfan and menazon to be quite safe for bees. Prakash and Kumaraswami (1984) evaluated toxicity of some insecticide against *A. cerana indica* and reported that by dry film, topical application and oral feeding methods, phosalone and endosulfan were found to be least toxic, monocrotophos and carbaryl were moderately toxic and the synthetic pyrethroids such as cypermethrin, permethrin, and decamethrin were most toxic to workers of *A. cerana indica*. Mishra and Verma (1987) evaluated the contact and stomach toxicity of some insecticides to *A. cerana* and reported endosulfan to be least toxic as contact poison than organophosphate, which showed more acute oral toxicity as compared to contact toxicity. Several workers have reported endosulfan as being safer to bees (Anderson and Wojtas 1986; Filipowicz 1982; Sorthia and Chari 1985), whereas, Lingappa et al. (1985) proved that bromophos was safer to bees than endosulfan while deltamethrin was more toxic than bromophos and endosulfan.

Presence of chlorinated pesticides residues in several insect species including *A. cerana* were noticed by Ahmad et al. (1987) and Andersen and Wojtas (1986). Buchler and Dreschler (1989) reported that when endosulfan was sprayed on different plant/crops, significant variation in the bee mortality due to application of endosulfan by different methods was observed. Singh et al. (1989) evaluated field toxicity of some insecticides to *A. cerana indica* and reported low toxicity of endosulfan and phosalone and high toxicity of dichlorvos, dimethoate, and malathion to honeybees.

In field trials conducted by Brar et al. (1992), fluvalinate was least toxic and had least residual activity as compared to carbaryl, endosulfan, and monocrotophos when sprayed on cotton. In Scotland, fewer incidents of bee poisoning associated with the use of fenitrothion on raspberries and gamma-BHC on oilseed rape were reported by Greig-Smith et al. (1994). Endosulfan was found to be most safe against aphid predator, *Coccinella septempunctata* and the honeybees, *A. cerana* when used for effective control of pest, *Dactyonotus carthami* (Singh et al. 1995). Endosulfan was reported to be more toxic than neem oil to *A. mellifera* (Panda et al. 1990; Abrol and Andotra 1997)

Rahman et al. (1997) evaluated the toxicity of certain insecticides to *A. cerana indica* and insecticidal residues in rapeseed and on the basis of toxicity, endosulfan (0.05, 0.10, and 0.15 %) was found to be moderately toxic with its toxicity persisting up to 48 h. The residues of malathion, cypermethrin, fenvalerate, and deltamethrin could not be detected in rapeseed but residues of endosulfan, at 0.10 and 0.15 % was detected at 0.002 and 0.005 ppm as endosulfan sulfate, respectively. Khan (2002) and Khan and Dethle (2004) evaluated eight pesticides against *A. cerana indica* by contact toxicity method and reported endosulfan as least toxic followed by Lambda cyhalothrin, alpha endosulfan, and imidacloprid while betacyfluthrin was highly toxic.

14.3.2 Toxicity of Organophosphate Insecticides

These are nerve poisons and affect cholinesterase activity and finally disturb the nervous coordination. These are the most widely used insecticides under tropical and subtropical conditions. Most of the organophosphates are broad spectrum and are highly toxic to honeybees as contact, stomach poison, and fumigants, both under laboratory as well as field conditions (Johansen 1961; Atkins et al. 1973; Hameed et al. 1973; Marsden 1979; Arzone and Patetta 1987; Thakur and Kashyap 1989; Sharma and Abrol 2005). Hameed et al. (1973) judged LC₅₀ value of insecticides after 24 h of treatment by exposing the bees to nectar of treated flowers of mustard crop and concluded that fenitrothion was most toxic followed by methyl demeton, phosphamidon, dimethoate, phosalone, carbaryl, and formothion. Based on the safety index, application of formothion, dimethoate, and phosalone at recommended rates were relatively safe to bees.

Kapil and Lamba (1974) compared the contact toxicity of 11 insecticides against foragers of *A. cerana indica* by direct spray method. Considering LC₅₀ value of

menazon (0.4916 %) as a unity, the toxicity ratios of endosulfan, formothion, methyl demeton, endrin, dieldrin, malathion, parathion, phosphamidon, lindane, phorate, and mevinphos were 1.17, 1.81, 14.0, 15.7, 17.79, 29.55, 26.01, 28.45, 32.65, 36.99, and 64.24, respectively. This indicated that menazon and endosulfan were safe. Bai and Reddy (1977) establish the order of toxicity based on speed with which all bees were killed. The organophosphate insecticides e.g., dichlorvos, methyl parathion, phosphamidon, and quinalphos were most toxic. Reddy and Reddy (1986) studied the effect of 10 organophosphates, 4 organochlorines, and 2 carbamates on the rate of oxygen consumption in *A. cerana indica* followed by topical application method. Results showed that all insecticides were inhibitory in action, and oxygen consumption decreased gradually with the progression of toxicity.

14.4 Symptoms of Poisoning

In laboratory studies, Kalita and Rehman (1995) found that monocrotophos and chlorpyrifos were most toxic to *A. cerana indica*, followed by oxydemeton methyl, neem oil, and deltamethrin. Vaidya et al. (1996) observed the repellent effect of insecticides to *A. mellifera* foraging on sprayed bloom of sarson. Malathion (0.05 %) was repellent for 5 days and fenvalerate (0.01 %) for 3 days, whereas endosulfan (0.06 %), quinalphos (0.05 %), dimethoate (0.03 %), methyl demeton (0.025 %), monocrotophos and phosphamidon (0.03 %) repelled bee for upto 4 days. Persistence repellency value indicated that fenvalerate, phosphamidon, and monocrotophos were the least repellent insecticides. Sharma and Abrol (2005) evaluated the contact toxicity of five insecticides applied to foragers of *A. mellifera* and *A. cerana indica* and reported that the toxicity of different insecticides after 8 h of treatment was in the order: malathion > cypermethrin > demeton-s-methyl > fenvalerate > deltamethrin for *A. mellifera* and malathion > demeton-s-methyl > cypermethrin > fenvalerate > deltamethrin for *A. cerana*. All the insecticides was highly toxic to bees.

14.5 Toxicity of Carbamates to Honeybees

Carbamates in general showed considerable toxicity to bees (Morse 1961a, b; Gromisz and Gromisz 1996). Under laboratory conditions, carbaryl was found to be toxic to bees as was DDT (Anderson and Atkins 1958a, b, 1966). Morse (1961a, b) reported mortality of bees due to carbaryl and stated that mortality was found high at 3 weeks after spraying. Carbofuran and carbosulfan were highly toxic to bees and it might cause severe losses if bees were present at the time or within few days after application of these insecticides (Bai and Reddy 1977). They also reported that carbaryl (0.31 %) is highly toxic to the bees *A. cerana indica* and caused mortality within 1 h of treatment. Seven-days-old bees were more tolerant to carbaryl than 21-days-old foragers (Higo 1980). Also, Egyptian–Iraqi hybrid bees were more tolerant

than native Iraqi bees (Mansour and Al-Jalil 1985). Danka et al. (1986) observed that European honeybee (*A. mellifera*) was more tolerant than African honeybee (*A. mellifera scutellata*) in terms of toxicity.

Suh and Shim (1988) evaluated different insecticides against *A. mellifera* and *A. cerana indica* by oral toxicity method. The LD₅₀ values of EPN, carbaryl, and malathion were in the order of 1.61, 1.99, and 4.32 ppm for *A. mellifera* and 0.75, 2.27, and 4.02 ppm for *A. cerana indica*. They showed significant difference between the enzyme kinetics of the two species. Oral application, using the recommended concentrations of the insecticides, revealed the following order: sevin > decis > mesothrin > cypermethrin. Finally, tests with residues on glass surface showed that sevin had the longest residual effect (up to 9 days after treatment) followed by decis, cybolt, mesothrin, and nurelle (Aillah et al. 1988).

Menon (1992) found that carbaryl and dimethoate caused far greater serious effect on brood rearing activity than BHC and cypermethrin. The residual toxicity and the insecticides affected the brood rearing activities of the honeybees. The brood rearing activity was considerably reduced during the first and second week after treatment. Abramson et al. (1998) evaluated the effect of insecticides; endosulfan, deltamethrin, cyfluthrin, and carbaryl on the learning activity of Africanized honeybee (*A. mellifera*) and reported that all the insecticides affected the learning to various degrees and that all the insecticides led to differing mortality.

14.6 Toxicity of Synthetic Pyrethroids to Honeybees

In modern era, pyrethroids are the most widely used insecticides against crops infested with insect pests. Acute toxicity tests in laboratory have shown most of these to be toxic through contact as well as oral routes (Benedek 1983; Kasamatsu 1986; Reith and Levin 1987). Mishra and Verma (1982) evaluated the acute contact and oral toxicity to Indian honeybee (*A. cerana indica*) and calculated LD₅₀ values of fenvalerate as 0.009 µg/bee (contact) and 1.712 µg/bee (oral).

Svendsen (1985) reported that permethrin and fenvalerate were toxic, endosulfan and phosalone were moderately toxic, while deltamethrin was less toxic to bees. Prakash and Kumaraswami (1984) reported cypermethrin, permethrin, and decamethrin as the most toxic insecticides. Fries (1985) found that cypermethrin 4.4 g a.i./ha and deltamethrin 7.5 g a.i./ha caused slightly higher mortality than that in control. Reduced pollen collection and residues of cypermethrin and deltamethrin was observed in the pollen of rape. Fenvalerate was highly toxic by ingestion method but slightly toxic by contact method (Arzone and Patetta 1982; Arzone and Vidano 1985; Somasundram and Raghupathy 1985). Mayer et al. (1984) found that fenvalerate drastically reduced the brood development however, the different groups of bees exhibited differences in their susceptibilities. Atkins and Kellum (1986) found that some brood poisoning may occur to larvae from fenvalerate at LD₁₀ level. However, at LD₅₀, it was 1.6-fold, and at the LD₉₀, 257-fold less toxic to brood than to adults.

The latter authors, however, did not report any deformities in emerging bees. They categorized it as a simple poison.

Cypermethrin was found highly toxic to bees (Delabie et al. 1985). Murray (1985) showed that WL-85871 (isomer of cypermethrin) and dimethoate were highly toxic to bees in topical and oral tests while in field tests, mortality of bees in case of WL-85871 was lower than dimethoate. Thakur et al. (1985) calculated LC_{50} values of 1–3-days-old honeybees and showed that on the basis of LC_{50} value, permethrin, cypermethrin, fenpropathrin, fenvalerate, and decamethrin were 13, 8, 8, 4, and 3 times, respectively as toxic as endosulfan. Atkins and Kellum (1986) reported that cypermethrin was more toxic to brood 1–2 days old than to adults. They further found that brood of 3–4 days old and 5–6 days old were approximately casually susceptible at LD_{50} and LD_{90} levels. Cypermethrin was a simple poison. Permethrin has been reported to be an approximately as toxic to larvae as to adults at LD_{10} level. However, at the LD_{50} and LD_{90} levels, it was 1.5-fold less toxic to brood than to adults. Permethrin acts as a simple poison. Since permethrin is a repellent which prevents bees from foraging on the treated crop, it is improbable that any of it would be transported to hive (Atkins and Kellum 1986).

Fenvalerate is reported to be less toxic ($LD_{50} = 0.23 \mu\text{g}/\text{bee}$) to the honeybee *A. mellifera* than deltamethrin (Kasamatsu and Kawachi 1986) and can be sprayed on many crops without any harm to honeybees. De Wael and Laere (1989) studied oral toxicity of the synthetic pyrethroids to honeybee (*A. mellifera*) under laboratory conditions. The LD_{50} values of fenvalerate, alphamethrin, and fenpropathrin were 0.425, 0.056 and 0.046 $\mu\text{g}/\text{bee}$, respectively. The tests on repellency showed that fenpropathrin was the most repellent followed by fenvalerate and alphamethrin. Egger (1989) found that a spray of the insecticide Decis (deltamethrin) was more effective than malathion or dimlin (diflubenzuron) against spruce sawfly (*Pristiphora betula*). Spraying during early morning did not kill the honeybees while foraging, however, 8 % of brood was dead in the colonies examined. Attalah et al. (1989) reported the Decis as the most toxic and sevin as least toxic insecticide in laboratory study. Hagler et al. (1989) reported that permethrin in combination with piperonyl butoxide was most toxic to bees. Inglesfield (1990) concluded that alpha-cypermethrin and vinchlozolin (a fungicide) sprays had no adverse effects on honeybee survival or colony development. Migula (1990) examined different techniques of application of insecticides to workers of honeybees in laboratory. It was found that mode of action differed with mode of application. At LD_{50} concentration of synthetic pyrethroids, metabolic disturbances were reversible and survival was due to detoxification of insecticides. Deltamethrin at LD_{20} did not affect mitochondrial oxidation.

Illarionov (1991) reported that alphamethrin, deltamethrin, cyhalothrin, cypermethrin, fenvalerate, bifenthrin, etofenprox, primiphos-methyl, and phoxim were highly toxic in the laboratory while fluvalinate exhibited low toxicity by ingestion and contact. Schmidt et al. (1991) tested cyfluthrin against honeybees and indicated that it was toxic, but probably because of repellency towards bees, there was no effect on brood. Deltamethrin, cypermethrin, methidathion, chlorpyrifos, and heptenophos were highly toxic to honeybees in laboratory conditions (El-Ansary and El-Zogby 1992). The LD_{50} values for these insecticides were 0.0074, 0.026, 0.21,

0.08, and 0.26 $\mu\text{g}/\text{bee}$, respectively. Estes et al. (1992) showed that permethrin residues were more persistent and caused 89 % mortality while in combination with chlordimeform, the bee mortality was 95 %. Jain and Shivrama (1993) evaluated the effect of pyrethroids on *A. mellifera* and *A. dorsata* and showed that fenvalerate was the safest and lambda cyhalothrin, the most toxic insecticide.

Bhuvneshwari and Uthamasamy (1994) showed that alphamethrin was more toxic to bees (*A. cerana indica*) than cypermethrin, endosulfan, and malathion. Kakar (1994) found that deltamethrin, fenvalerate, permethrin, and cypermethrin were extremely toxic to bees ($\text{LD}_{50} = 0.003\text{--}0.036 \mu\text{g}/\text{bee}$) on the basis of contact test. Lambda-cyhalothrin when applied in field had little effect on the presence of honeybees (Rotreki 1994). Wilde et al. (1995) showed that alphamethrin and deltamethrin had deleterious effect on bee mortality, colony development, brood area, and foraging by bees. Moreover, pesticide residues were also found in dead bee, honey, and pollen. Gromisz and Gromisz (1996) proved that betacyfluthrin was highly toxic and lethal concentration was 0.2–0.3 ppm/bee in oral test and 70 % mortality was observed at 0.03–0.12 % concentration in contact test. Talstar 100 EC (comprising 100 g a.i./L of bifenthrin) was proved to be highly toxic (Gromisz and Gromisz 1997). The contact toxicity test of this insecticide produced 80 % mortality. Erickson et al. (1997) found out the phase application of selected insecticide formulations on colony management and honeybee (*A. mellifera*) mortality. The foraging activity was reduced in all the insecticides. Permethrin caused less mortality than other insecticides. Khan (2002) evaluated the relative toxicity of some insecticides against *A. cerana indica* and found betacyfluthrin as most toxic and endosulfan as least toxic (Khan and De-the 2004). Haarmann et al. (2002) studied the potential impacts of fluvalinate and coumaphos on the honeybee *A. mellifera* queen's viability and health. The queens treated with high concentrations of fluvalinate weighed significantly lesser than those treated with low concentrations or control, but otherwise appeared to develop normally. Queens treated with coumaphos showed sublethal effects, including physical abnormalities and atypical behavior. The queen weighed significantly lesser than the control. Sharma and Abrol (2005) established contact toxicities of malathion, demeton-s-methyl, cypermethrin, fenvalerate, and deltamethrin and reported that all the synthetic pyrethroids were highly toxic, cypermethrin being the most toxic to both *A. mellifera* and *A. cerana*.

14.7 Neonicotinoids' Effects on Bee Poisoning

Populations of honeybees and other pollinators have declined worldwide in recent years. A variety of stressors have been implicated as potential causes, including agricultural pesticides. Pollinator health is receiving increased attention as both managed pollinator (i.e., honeybees) and native pollinator populations decline worldwide (van Engelsdorp et al. 2007, 2008; Biesmeijer et al. 2006). Several causal mechanisms (including viral pathogens, parasitic mites, and pesticides) have been proposed and

investigated as contributing causes (Alaux et al. 2010). Pesticide exposure has received significant attention and recently published analyses of pollen from managed bees located near agricultural environments demonstrated that many agricultural chemicals (including insecticides, miticides, fungicides, and herbicides) are detectable in honeybee wax and pollen samples (Mullin et al. 2010; Wu et al. 2011). Of the many compounds detected, the neonicotinoid group has arguably received the most attention. These compounds act as nicotinic acetylcholine receptor agonists in insects, causing persistent excitation of these receptors and eventually death (Jeschke and Nauen 2008). As a group, neonicotinoids possess several key attributes that have facilitated heavy adoption in both agricultural and urban environments, including low vertebrate toxicity (US Environmental Protection Agency 2003) and the ability to be translocated by plants. Neonicotinoids are also persistent, offering the potential for a large window of activity. They include the chemicals imidacloprid, clothianidin, fiprinol, acetamiprid, thiacloprid, thiamethoxam, dinotefuran, and nitenpyram. Neonicotinoids are a type of insecticide, differing from conventional spray products in that they can be used as either seed dressings or as soil treatments and as a result, are dispersed into plant tissues resulting in a slower (chronic) exposure to nontarget organism. The half-lives of these compounds in aerobic soil conditions can vary widely, but are best measured in months (148–1,155 days for clothianidin) (US Environmental Protection Agency 2003).

This set of insecticides has become an increasing concern to beekeepers and bee researchers, with many suspecting that neonicotinoids may be connected to current bee declines which has led to either full or partial ban of some of these chemicals in a number of European countries, including France, Germany, Italy, and Slovenia; and a large body of research investigating the issue (Thompson and Maus 2007).

Krupke et al. (2012) reported that neonicotinoid insecticides found in honeybee pollen and comb material are highly toxic to honeybees, but the routes of their exposure have remained largely undefined. They analyzed samples of honeybees and pollen stored in the hive and found that during spring, extremely high levels of clothianidin and thiamethoxam were found in planter exhaust material produced during the planting of treated maize seed. They also found neonicotinoids in the soil of each field sampled, including unplanted fields. Plants visited by foraging bees (dandelions) growing near these fields were found to contain neonicotinoids as well. This indicates deposition of neonicotinoids on the flowers, uptake by the root system, or both. Dead bees collected near hive entrances during the spring sampling period were also found to contain clothianidin, although whether exposure was oral (consuming pollen) or by contact (soil/planter dust) is unclear. They also detected the insecticide clothianidin in pollen collected by bees and stored in the hive. When maize plants in their field reached anthesis, maize pollen from treated seed was found to contain clothianidin and other pesticides; and honeybees in their study readily collected maize pollen. These findings clarify some of the mechanisms by which honeybees may be exposed to agricultural pesticides throughout the growing season. These results have implications for a wide range of large-scale annual cropping systems that utilize neonicotinoid seed treatments.

Krupke et al. (2012) after sampling anthers directly and identifying maize pollen in their samples, found that pollen originating from treated seed does contain clothianidin, although not at the levels found in some of the bee-collected pollen samples, indicating the likelihood of additional pathways or sources. The levels of clothianidin in bee-collected pollen was approximately tenfold higher than that reported from experiments conducted in canola grown from clothianidin-treated seed (Cutler and Scott-Dupree 2007). Detection of clothianidin in pollen, both in stored pollen in cells and in pollen traps is a critical finding because clothianidin is even more toxic when administered to bees orally, with an LD50 of 2.8–3.7 ng/bee (Laurino et al. 2011; US Environmental Protection Agency 2011).

The latter authors also detected three fungicides in bee-collected pollen samples (trifloxystrobin, azoxystrobin, and propiconazole). Azoxystrobin and trifloxystrobin are frequently used in maize seed treatments as protectants and all three of these compounds are also widely applied to maize in North America, even in the absence of disease symptoms (Paul et al. 2011). These compounds are typically applied using aerial application during anthesis. Propiconazole has been shown to synergize toxicity of some neonicotinoids (thiacloprid and acetamiprid) to honeybees in the laboratory, although the same results have not been shown in field studies (Iwasa et al. 2004; Schmuck et al. 2003). Although these fungicides are not acutely toxic to honeybees (Mullin et al. 2010), the fact that they are routinely applied to areas that bees will frequent (i.e., maize plants at anthesis) coupled with the difficulties and uncertainties in assessing the toxicity of pesticide mixtures (Lydy et al. 2004), indicate that they should be considered in future work.

In recent years, the major shift in agriculture has been the development and extensive deployment of neonicotinoid and phenylpyrazole pesticides. The phenylpyrazoles, including fipronil, bind to γ -amino butyric acid (GABA)-gated chloride ion channels and block their activation by endogenous GABA, leading to hyperexcitation and death (Gunasekara et al. 2007). Fipronil acts by contact and stomach action on the insects. It is effective against a variety of pests, but there are increasing concerns about its environmental and human health effects. Its use has become problematic in France, where it has been proven responsible for the drop in bee population, after bees became disoriented and unable to return to their hives. It is one of the main chemical causes blamed for the spread of “colony collapse disorder” among bees. It has been found by the Minutes-Association for Technical Coordination Fund in France that even at very low nonlethal doses for bees, the pesticide still impairs their ability to locate their hive, resulting in large numbers of forager bees lost with every pollen-finding expedition.

Neonicotinoid and phenylpyrazole insecticides differ from classic insecticides in that they become systemic (Trapp and Pussemier 1991) in the plant, and can be detected in pollen and nectar throughout the blooming period (Cutler and Scott-Dupree 2007). As a consequence, bees can experience chronic exposure to them over long periods of time.

While some studies have shown no negative effects from seed-treated crops (Nguyen et al. 2009), acute mortality was the only response measured. Desneux et al. (2007) examined methods that could be used to more accurately assess the

risk of neonicotinoid and phenylpyrazole insecticides including a test on honeybee larvae reared in vitro to test for larval effects (Aupinel et al. 2005), a proboscis extension response (PER) assay to access associative learning disruption (Decourtye and Pham-Delegue 2002), various behavioral effects (Thompson 2003), and chronic exposure toxicity beyond a single acute dose exposure (Suchail et al. 2001; Decourtye et al. 2005; Ailouane et al. 2009). Pesticide exposure may interact with pathogens to harm honeybee health. Honeybees that were both treated with imidacloprid and fed *Nosema* spp. spores suffered reduced longevity and glucose oxidase activity (Alaux et al. 2010).

The seed-dressing for sunflowers and maize has worsened the problem with increased honeybee deaths from 1994 onwards in France (Nauen et al. 2001). There are at least two levels of toxicity, one centered at about 5 ng/bee and the other one at around 40 ng/bee. Moreover, imidacloprid and its metabolites would still be very toxic at much lower doses due to chronic intoxication. Briefly, studies converge and show a complex mechanism with an important toxicity at doses of imidacloprid in the $\mu\text{g kg}^{-1}$ range, and even for lower amounts (Colin and Bonmatin 2000; Colin 2001; Guez et al. 2001; Belzunces 2001).

Bonmatin et al. developed analysis by high performance liquid chromatography coupled with mass spectrometry in tandem (HPLC/MS/MS) to detect and quantify imidacloprid in soils, plants, flowers, and pollens with a limit of detection (LOD) of $0.1 \mu\text{g kg}^{-1}$ and a limit of quantitation (LOQ) of $1 \mu\text{g kg}^{-1}$ (Bonmatin et al. 2000a, c, 2001). They found the presence of imidacloprid in pollens with average values of $3 \mu\text{g kg}^{-1}$ (sunflowers and maize). Thus, imidacloprid appears to be bioavailable for bees in fields in a range of concentrations corresponding to that of sublethal effects on bees and especially concerning the foraging activity (Colin and Bonmatin 2000; Colin 2001). This risk situation with respect to sunflowers and maize is worsened when considering (i) the additional toxic action of several imidacloprid metabolites (Nauen et al. 1998; Oliveira et al. 2000) as well as (ii) the very low concentrations inducing chronic mortality of bees which are in the $0.1\text{--}1 \mu\text{g kg}^{-1}$ range (Suchail et al. 2001; Belzunces 2001). The commercialization and the use of Gaucho® on sunflowers have been suspended in France since 1999 (Schmuck et al. 2001).

14.8 Predicting Honeybee Mortality

In a Leaflet 2883 (Malcolm T. Sanford), Division of Agricultural Sciences, University of California, 1981, were mentioned devised ways to predict honeybee mortality in the field when the pesticide is applied as an early morning spray. In most instances, the LD_{50} (the experimental dosage at which 50 % of a test bee population died) in micrograms per bee ($\mu\text{g/bee}$) can be directly converted to the equivalent number of pounds of chemical per acre when applied as a spray to the aerial portions of plants. (For kilograms per hectare (kg/ha), multiply (mgr)g/bee by 1.12). For example, since the LD_{50} of parathion is $0.175 \mu\text{g/bee}$, we would expect that 0.17 lb/acre (0.2 kg/ha) of parathion would kill 50 % of the bees foraging in the treated field at

the time of application or shortly afterwards. Generally speaking, some pesticides can be made safer to honeybees by slightly lowering the dosage. Conversely, by increasing the dosage only slightly the pesticide may become highly hazardous to bees. This information is particularly useful when the LD_{50} in micrograms per bee is approximately equal to the normal dosage in pounds per acre needed in the field to control pest populations. For example, consider a pesticide which is normally applied at dosages of 0.5 to 1.5 lb/acre, having an LD_{50} of 1.0 $\mu\text{g}/\text{bee}$. Furthermore, suppose that the pesticide has a slope value of 4.0 probits. Then, if this chemical is applied at 0.5 lb/acre, we would expect a 12 % kill of bees; at 1 lb/acre, we would expect a 50 % kill; and, at 1.5 lb/acre, we would expect a 72 % kill. It is emphasized that the method described is a rule-of-thumb, and that some pesticides are more or less hazardous than one can anticipate from the laboratory data. Most of these are pesticides which have very short or very long residual characteristics.

14.8.1 The Honeybee Mortality Predictor: A Rapid Method

The nomogram provides a quicker method of predicting the mortality of honeybees from field applications of pesticides which requires mathematical calculations. The method is also useful for predicting potential hazards to honeybees when applying pesticides for mosquito abatement and for pest control in forest, rangeland, recreational areas, and home gardens. How this predictor model works can be explained from the following example. If parathion has an LD_{50} of 0.175 $\mu\text{g}/\text{bee}$, slope value of 4.96 probits and we intend to apply parathion at the rate of 0.25 lb/acre a.i. (active ingredient) to control pest populations of insects in an area which contains colonies of honeybees for pollinating the crop, how hazardous will this dosage be to the bee colonies if they cannot be protected during application or removed to safety? From the model it is predicted that application of 0.25 lb/acre of parathion will kill approximately 78 % of the bees that contact the treated foliage or that are flying through the treated area during the application of the spray. The bee mortality would be reduced to approximately 50 % (to 39 % in the example) if the parathion application was made during the night (from darkness to 4 a.m.), and bee mortality would be increased approximately two times if the parathion application was made after 7 a.m. and later into the day (more than 98 % in the example). Also notice that by reducing the dosage only slightly to 0.22 lb/acre, the mortality is reduced from 78 to 65 %.

Tropical and subtropical climate of India in particular presents suitable conditions for the outbreak and appearance of many pest problems. The pest problems have been further aggravated by the advancement in agricultural technology. Irrigated crops, intensive agriculture, introduction of crops and crop varieties, and disturbing the indigenous and primitive cropping patterns have contributed in increasing the pest problem of crops. Reduction in uncultivated land, corners, and bunds destroy nesting and hibernating places of wild pollinators and succession of nectar and pollen yielding flowers round the year is destroyed. Weedicides are used to control the weeds and hence lead to starvation of pollinating insects. The advanced agricultural technology

has helped to destroy the agriculture cycle through indirect effect. There is also a prominent negative direct factor, i.e., the insect pollinators are killed by pesticidal usage in crop protection. There is increasing use of pesticides for the control of rodents, mites, insects, nematodes, and fungal and bacterial diseases of crop plants. The loss by bee kill is direct, i.e., loss of honey production and indirect inadequate pollination of crops resulting in reduced productivity.

Integrated pest management in India has not yet shown desired results and blanket pesticidal applications are given. Most farmers apply large quantities of pesticides at regular intervals and in most cases the pesticides are nonselective coupled with untimely application. Unfortunately honeybees are susceptible to many pesticides used in pest control programs. This problem is recently overshadowing all other problems in apiculture. Farmers in India have small holdings and hand sprayers/dusters are commonly used for treating small areas each day. This results into a continuous threat of chemical poisoning to bees. Moreover, there is no coordination between the beekeepers and the farmers by any Government decree and therefore, measures to save bees cannot be taken.

Large number of killed bees is found in front of the hives or in the fields by insecticidal poisoning. It is not possible to quantify the loss in terms of food production or to assess the financial value of the bees killed. Even more important is the loss in future crop yields because a beekeeper whose bees are killed gives up beekeeping and others too are discouraged to take up beekeeping. Therefore, a balance sheet between the gains in crop yields by control of pests and losses due to decreased pollinator activity and honey production by bee kill should be worked out. While controlling pests the scientists and farmers are looking on to one aspect of the economic considerations in insecticidal applications. Our primary aim should be to assess how crop pests can be kept under control without killing insect pollinators and to ensure optimum pollination by these insects. Widespread destruction of beneficial insects (including pollinators) often occurs as a consequence of irresponsible and improper use of pesticides. It should be accepted that some loss is inevitable in certain circumstances and that a realistic aim should be an acceptably low level of loss rather than complete protection of bees.

Some insecticides have been screened in laboratory in India for their toxicity to bees. The first study in this field was carried out by Cherian and Mahadevan with DDT and gammaxene against *Apis cerana indica*. Hameed allowed the worker bees of *A. mellifera* to forage on cut flowers of mustard to which systemic and contact insecticides had been sprayed. On the basis of safety index, formothion, vamidothion, dimethoate and phosalone were considered to be relatively safer to bees. Contact toxicity to *A. cerana* of insecticide applied as sprays was compared with Menazon by Kapil. Taking the LC_{50} for Menazon as 100, the comparative values for endosulfan, eormothion, methyl demeton, endrin, dieldrin, malathion, parathion, phosphamidon, lindane, phorate, and mevinphos were 1.17, 1.18, 14.00, 15.70, 17.79, 22.25, 26.01, 28.45, 36.99, 57.96, and 64.24 respectively. Singh tested 15 insecticides and reported that menazon and endosulfan were least toxic and were considered nontoxic to *A. cerana* at their recommended doses. According to Thakur et al. (1981), fenitrothion and fenthion were highly toxic as compared to endosulfan

and trichlorfon and hinosan were moderately toxic as determined by residue film method. Comparative toxicity of organophosphates, chlorinated hydrocarbons, and carbamates was worked out by Bai Attri who also assessed the contact and oral toxicities of some insecticides. Toxicity of several organophosphates to *A. cerana* was determined in the laboratory using topical application method. Determination of the kinetic parameters of the reactions by the authors showed that differences in anticholinesterase activity were due mainly to differences in affinity rather than different chemical structure of the compounds.

Cholinesterase inhibition by insecticides in Indian honeybee was studied by Dale Bai who reported that signs of poisoning in *Apis cerana indica* were first observed when acetylcholinesterase inhibition exceeded 35 % and death occurred at 96 % or more inhibition.

Reddy reported the inhibition of magnesium-activated adenosine triphosphate as the criterion to determine the degree of organochlorine insecticide poisoning to *Apis cerana indica*. Digestive amylase and protease of *Apis cerana indica* were inhibited to the same level by the insecticide poisoning from different groups of insecticides. Studies on the level of ions of amino acids in the hemolymph of worker bees of *Apis cerana indica* treated topically showed pronounced stimulatory effect with organophosphates, a relatively strong inhibitory action with chlorinated hydrocarbons, and an intermediary effect with carbamate pesticides.

Bee poisoning or killing of bees from pesticides continues to be a serious problem for beekeepers. Most bee kill occurs when pesticides are applied or allowed to drift on to blooming crops or weeds (Mayer 2003). Most (99 %) bee kills result from bees picking up the pesticides when foraging. The hazards of insecticidal application on flowering crops include—direct mortality, fumigative effects, repellent effect, and toxicity of residues present on various floral parts and in nectar to the insect visitor. A highly toxic insecticide generally reduces the field force of a colony within a short period of time. Colonies may be reduced by one third to half in strength within 24–48 h (Eckert and Shaw 1960), thus adversely affecting both the production and marketing segments of the honey and beekeeping industry. Generally, fumigative action of insecticides used under field conditions is of much shorter durations than the effect of contact and stomach poison. A prolonged repellent effect will deprive the flowers of pollination benefits of insect visits, while a short repellency will deter the insect pollinators from visiting the treated bloom for a brief period and thereafter, allow them to resume their foraging activity (with minimum residual hazards) without compromising with the yield potential of the crop.

Poisoning of insecticides to honeybees is generally more pronounced because of their long hours of working on the crop flowers for pollen and nectar collection, continuous working in nature, and long flights with pollen loads. Bee poisoning is more chronic in areas which lack sufficient wild pollens and nectar plants to sustain the number of colonies required for crop pollination. Most bees are pastured in agricultural areas where they are subjected to killing from pesticides (Mayer and Johansen 1983). The beekeeper has little or no control of when and what pesticides are applied in the areas of his bee forage (Mayer 2003).

Conservation of honeybees for crop pollination is vital to agricultural production (Bisht et al. 1983; Sorthia and Chari 1985; Smirle et al. 1990; Gupta 1994; Dhuley et al. 1996; Russel et al. 1998., Kremen et al. 2002).

Many colonies that are not killed outright may be weakened to such an extent that they are no longer effective as pollinators or honey producers, nor can they be divided to increase the number of colonies. This type of economic loss far exceeds the loss from colonies being killed outright by pesticides. Though the monetary loss to the beekeeping is large, yet the value of the seed and fruit lost through the lack of pollination is estimated to be 50–100 times greater (Atkins 1975).

Pesticide application may also change the physiology of nectar and pollen producing plants, the attraction of bees to flowers, affect pollen viability, and reduce pollen germination on contaminated stigma (Eaton 1963; Church and Williams 1978; Fell et al. 1983; Kuriachen et al. 1992; Sharma 1993). All these effects of pesticides usages are serious to pollination potential and honey production.

The immature stages of honeybees are vulnerable to insecticidal poisoning that result in hidden damage to honeybee colony (Davis and Shuel 1985; Naumann and Isman 1996). Loss of brood and new bees as result of exposure of insecticides may be more deleterious than the loss of foragers (Abrol and Kumar 2000a, b; Abrol and Kumar 2001; Abrol and Sharma 2007) because the latter could be replaced more quickly and provide less potential value to the colony than the emerging workers.

The problem of bee poisoning is as old as about 1870s when Thompson (1881) detected accidentally killed bees by application of Paris green to pear trees in bloom and some sort of bee malady was found around beehives at that time. The intermediary period between 1870 and 1888 contributed further to the knowledge of the toxicity of Paris green and London purple. Brose (1888) demonstrated that these insecticides in sugar syrup repelled the bees to some extent but those which fed on syrup were killed in 1–4 h. Troop (1918), Hoskin and Harrison (1934) reported some inorganic compounds like arsenic highly toxic to honeybees. Likewise, Kingsmill (1917) reported accidental killing of bees to a great extent from Paris green and molasses bait mixture. Bourne (1927) reported that blossom treated with a mixture of lead arsenate, lime sulfur, and nicotine sulphates were unattractive to bees.

Many major agricultural changes took place in the 1950s, shortly after World War II, when tractors replaced horses, chemical fertilizers replaced organic manure, aerial application of pesticides became common place, and farmers became increasingly conscious of business costs. At the same time, many farmers were encouraged to devote large acreages to the cultivation of a single crop, which necessitated the utilization of large quantities of synthetic fertilizers and pesticides to nourish and protect that crop. Consumers also came to expect all market fruits and vegetables to be completely free from insects and insect damage.

Thus, many growers found it advantageous to apply more and more pesticides each year. Unfortunately, some aspects of this agricultural modernization were not beneficial for beekeepers, whose needs were either frequently forgotten or ignored. Consequently, many honeybees were killed. To compensate, many commercial beekeepers had to keep larger numbers of honeybee colonies in a variety of locations to make up for losses from pesticides and to meet rising operating expenses. This

interaction between the needs of crop farmers and the needs of beekeepers, coupled with frequent widespread application of the “newer” insecticides such as parathion, proved devastating to thousands of colonies.

14.9 Factors Influencing Bee Poisoning

By nature, honeybees from a colony visit flowers over an area of several square miles. The intensity of visitation in any one part of the area is determined by the relative attractiveness of the flowers. The extent of damage to the colony by a pesticide application is influenced not only by the relative toxicity of the material, the number and methods of application, the time of day, and the weather conditions, but also by the number of bees from the colony visiting the flowers in the treated area, the type of food (nectar or pollen) they are collecting, the type of flowers the food is collected from, the season of the year the damage occurs, and even the influence of forage available to the bees for weeks before and after the application. Wild bees are also damaged by pesticides. Poisoning may result from contaminated food as well as from florets, leaves, soil, or other material used by the bees in nesting. The toxicity of a specific insecticide to honeybees and wild bees is not always the same, and even among wild bees some materials are more toxic to one species than to another. Different factors influencing bee poisoning are as given below:

14.9.1 Factors Affecting the Severity of Poisoning

14.9.1.1 Environmental Factors

Temperature In the case of organophosphorous or carbamate compounds, the higher the temperature, the more toxic is the compound. The converse is true for pyrethroids and cyclodienes.

Humidity, Light The toxicity of various organophosphorous compounds is positively correlated with humidity. Light influences the activity of many insects, including bees, and thereby affects their exposure to poisoning.

Chemical Degradation Some metabolites of the active ingredient can be more toxic than the original compound. A well known example is the oxon form of some organophosphorus compounds.

14.9.1.2 Factors Dependent on Application

Formulation The distribution of the toxin in the environment, its stability, its penetration into the insect, and its metabolism depend primarily on the type of formulation.

Mixture and Synergy In order to reduce the number of field applications, farmers often mix different types of agrochemicals. If the formulations are chemically compatible, a synergistic effect occurs in some cases, greatly increasing the toxicity of each individual component of the formulation. This synergy was defined by Macht (1929) as “the phenomenon exhibited by the combination of two or more drugs in which the pharmacodynamic effect produced by the mixture is not a simple summation of the effects produced by the two or more individual components. Such combinations produce a pharmacological effect of an unexplained nature in that the effect of one component may be greatly heightened or potentiated by the other”.

Concentration Generally, the more concentrated the formulation, the more toxic is its effect. However, in the case of per os penetration, some insecticides can be repellent at high concentration.

Exposure Time This depends on the mode of application, on the environmental stability of the formulation and on the accessibility of the treated surfaces to bees. Puddles, present in the field before spraying or created by run-off when it rains just after spraying, can be contaminated with agrochemicals and provide a source of toxins when bees need water.

Droplet Size and Nature As discussed earlier, toxicity is inversely correlated with the diameter of the droplets and oil formulations are generally more toxic. The means of penetration through the respiratory ducts can become important if the droplet diameter is less than 10 microns. Moreover, aerosols can be dispersed by the wind and reach nontarget crops.

14.9.1.3 Factors Dependent on the Insect

Age There is great variability in the effect of agrochemicals on bees which depends not only on the type of active ingredient but also on the life stage or age. It is often difficult to distinguish between adult bee mortality due to natural ageing or exhaustion due to intense foraging activity and mild poisoning. Newly emerged bees are the most susceptible to DDT and carbaryl. By contrast, older bees are more susceptible to malathion and methylparathion. The youngest stages of larvae are generally the most susceptible.

Nutrition Starvation significantly increases susceptibility to toxins, probably because the fat bodies, which are important sites of detoxification, are considerably reduced in size under such circumstances.

Sex and Caste The primary role of drones is to mate with virgin queens. Excluding their reproductive role, their contribution to the life of the colony is considered negligible. By contrast, the queen fulfils an essential role as the only reproductive female. Adequate nutrition of the queen is essential to enable her to lay up to 2,000 eggs a day, but the particular susceptibility of queens to toxins is not known.

Resistance Bees may have the ability to develop resistance to certain insecticides.

Group Effect This effect was first described in *Aedes aegypti*. The greater the number of insects in a defined volume, the greater the susceptibility of individuals to the toxin. Although similar effects have not been demonstrated for bees, one can imagine that 5,000 adult bees per liter is a very high concentration of insects. In conclusion, the additive action of each individual factor which is harmful for the insect, can increase the toxicity more than 100 times.

14.9.1.4 Pesticide Formulation

Dust is highly hazardous to bees because of its tendency to drift to considerable distance; particles remain adhered to plant surface for long. So is the case with wettable powders which also remain unabsorbed on the plant surface for longer periods than emulsifiable concentrates. The emulsifiable concentrates are relatively safer (Kapil 1970). The granular insecticides are safest. However, granular insecticides with systemic action may contaminate nectar and may result in losses to bees, foraging upon them. The insecticides with fumigant effect may also be hazardous to bees. Microencapsulated granules applied on flowers are sometimes collected by bees and stored in the hives, where they may be eaten by adult bees or fed to brood causing high mortality besides causing long term contamination of hive parts and hive products.

14.9.1.5 Selectivity of Pesticides

There are some insecticides which have little effect on honeybees when applied as sprays e.g., endosulfan, phosalone, pirimicarb, fluvalinate, or trichlorfon. High toxicity of carbamates to honeybees has been considered due to their very low level of phenolase enzymes. It has also been claimed that greater acetylcholinesterase concentration in young enables them to tolerate malathion. Similarly, high tolerance of trichlorfon by honeybees has been correlated with relatively high pH of the body.

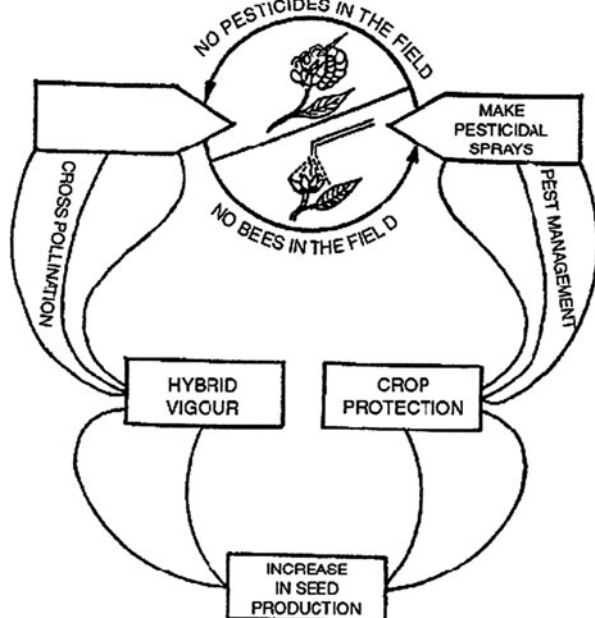
14.9.1.6 Period of Application

Insecticides when applied to flowering crops or pesticides when applied to a non-flowering crop but having large number of attractive flowering weeds or hedges in the fields or in the adjoining fields may be hazardous to bees. The bees are also affected if they pass through a field treated or sprayed with pesticides. In mango orchards bees attracted to honeydew secreted by mango hoppers are killed in large numbers when insecticides have been applied to the trees.

14.9.1.7 Time of Application

Little foraging occurs early in the morning or late in the evening. Application of pesticides during late evening or early morning provides relative safety. This avoids direct deposition of pesticides on the bee body and even residues on the treated surfaces

Fig. 14.1 Schematic model exhibiting timing for application of control measures for safety to bees



are rendered less harmful, especially in case of short residual pesticides. Species variation has also been noticed. *A. cerana* became active at 7–10 °C ambient temperature, whereas *A. mellifera* never became active unless the ambient temperature reached 13–16 °C or above (Fig. 14.1).

14.9.1.8 Attractiveness of Crop

Some crops notably rapeseed and mustard are extremely attractive to bees which will forage from colonies upto 3 km away or more. These crops remain attractive until the very end of flowering and even in cool and dull weather when the bees do not visit other crops.

14.9.1.9 Weather

Warm and sunny weather is conducive for foraging by bees. When there is a prolonged dull weather the foraging activity is reduced considerably and insecticides can be applied on the crop.

14.9.1.10 Temperature

Temperature is probably the most significant factor causing differences in the toxicity of pesticides. Immediate effects may be much greater at higher temperatures whereas,

residual effects are likely to be less because the toxic materials breaks down more quickly.

14.9.1.11 Method of Application

Aerial application of pesticides has been regarded as more hazardous than ground application because bees get less time to escape the drift of insecticides. Systemic insecticides applied on the blooming crop may cause hazards to the bees. Fine sprays are safer than coarse sprays.

14.9.1.12 Colony Strength

Populous colonies always suffer greater losses than small colonies, because more foragers are exposed to insecticides.

14.9.1.13 Age and Body Size of Honeybees

Newly emerged bees are more susceptible to insecticides than older bees. Smaller bees likewise are more susceptible to insecticides because their body surface area is larger in relation to their body weight.

14.9.1.14 Distance of Colonies

Honeybee mortality is inversely proportional to the distance of colonies from treated fields. Farther the crop from colony, less likely is it to attract large number of foragers.

14.10 How Bees are Exposed to Pesticidal Hazards

Many of the crop plants need cross-pollination. At the same time they are infested by pests even during flowering causing considerable losses which warrant the application of control measures. The pesticidal applications pose serious danger and eliminates large population of insect pollinators as well. Some of the crops benefiting from bees as well as heavily attacked by pests include:

14.10.1 Cotton

It is the most dangerous crop for bees. As many as 15–20 insecticidal applications at shorter and regular intervals are recommended for the control of various cotton

pests. The flowering continues for about 2 months and during this period insecticides are regularly applied for the control of many pests like bollworms, aphids, bugs, etc. Foraging bees are killed by these sprays. New generation of bees develop in 3 weeks. Insecticidal applications at shorter intervals than this kill more adult bees than can be replaced and ultimately the colonies die. However, coordinated application of insecticides can minimize bee kill. (1) Flowering in cotton continues for about 2 months but flowers that set fruit appear within 3–4 weeks. Therefore, use of insecticides during this period should be reduced so that bees can be moved to the crop. (2) Nectar in flowers and extra floral nectaries is exhausted by mid-day and very few bees are foraging in the afternoon when insecticides can be applied with reduced hazards to bees. (3) Air spraying has picked up for cotton. In such a situation, the colonies should be located away from the flight path of the plane.

14.10.2 Brassica and Vegetable Seed-Crops

These are attacked by aphids, caterpillars, and bugs during flowering and pod formation stage. These crops include oilseeds Brassica, seed crops of cauliflower, cabbage, raddish, turnip, carrot, fennel, and coriander. In these crops too, the flowering is greatly extended, lasting for about 1–1 ½ months. These crops need insecticidal applications during flowering periods. But all these crops are also enthusiastically foraged by bees which are very useful pollinators of these crops. There is extensive pesticidal poisoning to bees on these crops. There are no specific recommendations to safeguard bees and only general guidelines to reduce bee kill can be followed, though Singh (1969) sprayed endosulfan on mustard to control aphids at 0800, 0900, 1630 or 1730 hours and found that *Apis* spp. foraged between 1030 and 1530 hours without any effect on foraging intensity and no bees were killed.

14.10.3 Sunflower

Its cultivation is gaining importance in India. Bees contribute much in increased crop production by pollination services but bee losses have been reported by insecticidal sprays for the control of aphids and caterpillars. In India, endosulfan was found to be less toxic to honeybees than fenthion, carbaryl, or parathion and seed set and yield were not affected since bee activity was not reduced in endosulfan sprayed plots (Ramakrishna et al. 1974; Bhattacharya et al. 1982). Bees mostly forage in the forenoon and there is limited activity till early afternoon. Therefore, evening or late in the afternoon is appropriate time for chemical control operations.

14.10.4 Sesame

It is automatically self-pollinated but natural cross-pollination also occurs. Honeybees are very active on the flowers of sesame. The crop at flowering stage suffers from

the attack of aphids, brown leafhopper, sucking bugs, whiteflies, and caterpillars. Chemical application at blossoming would cause hazards to bees also.

14.10.5 Seed Crops

Seed crops like lucern and clovers are rich bee forages. Under semi-arid tropics, the legume flowers usually close in the afternoon and it allows time for safe application of pesticides afterwards against caterpillar pests.

14.10.6 Pulses

Pulses like soybean, cajanus, and others are self-pollinated crops but yield increases by bee pollination have been observed. Considerable mortality of honeybees from insecticide poisoning is reported in some countries but lack of such reports from India is due to non-monitoring of hazards.

14.10.7 Cucurbits

These require the control of fruit flies, pumpkin beetle, and aphids when in flowering. Cover sprays of insecticides are given against these pests. Honeybees visit the flowers of melon and other cucurbits. Steps to minimize bee kill from these sprays are required.

14.10.8 Tobacco

In tobacco, self-pollination is normal but honeybees and other insects visit the flowers for nectar, affecting some cross pollination. Aphids, whiteflies, thrips, and caterpillars are the pests which may warrant insecticidal application during flowering which consequently would lead to bee hazards.

14.10.9 Coffee

Flowering period in coffee is short and insecticidal applications can be avoided during coffee flowering. Coffee may be attacked by bugs, leaf miner, and thrips during flowering. In case of outbreak during flowering, the crop should be treated when bees are not active and less persistent insecticides be used.

14.10.10 Pome and Stone Fruits

Apple, peach, plum, apricot, and almond are attacked by caterpillars at blooming time. Insecticidal use has been suggested by economic entomologists against blossom thrips, though economic losses by thrips have not been ascertained. The recommendations are made in ignorance of insecticidal bee hazards. Therefore, caution is important so that the huge benefits from bee pollination are not reduced.

14.10.11 Other Fruit Crops

Insecticides can be applied for pest control at flowering time in citrus, litchi, olive, grapes, coconut, and cocoa. Care should be taken because they are also visited by bees for floral rewards.

14.10.12 Phytotoxicity to Plants

The effect of an insecticide application may not be confined to damage to the pollinators of a distant crop or elimination of pollinators for the target crop. Another previously overlooked factor associated with the pesticide may be that it can detract from the plants' productiveness. Sedivy (1970) reported that only 10.5 % of pollen grains germinated after they were dusted with Melipax (Melipax is a toxaphene-like chlorinated camphene) as compared to 62.1 % in the control pollen. When the pollen grains were treated with 0.3 % Fribal emulsion, another apparently toxaphenelike compound, only 28.2 % germinated as compared to 81.5 % of the control pollen. None of the grains treated with 0.7 % Fribal emulsion germinated as compared to 79.0 % of the control. Gentile et al. (1971) reported that the insecticide naled, at only 100 ppm, completely inhibited germination of both tomato and petunia pollen. They also reported that azinphosmethyl, DDT, dichlorvos, dicofol, endosulfan caused reduction in pollen germination and/or pollen tube elongation. Carbaryl and methomyl had little or no deleterious effect on pollen, and xylene was noninjurious. The separation of the toxic or repelling effect of the presence of the insecticide on the plant from the possible less attractiveness of affected pollen is difficult, but the idea merits further examination, both from the effect of pesticides on the plants and on the pollinating insects.

14.11 Intensity of Damage to Bees by Pesticides

Numerous surveys have been made to determine the extent of the losses of bees from pesticides. Levin (1970) stated that some 500,000 colonies were killed or damaged in the USA in 1967, of which 70,000 were in Arizona and 76,000 in California.

Swift (1969) stated that losses in California in 1968 were even greater—83,000 colonies. Wearne et al. (1970) and Barnes (1972) concluded that the major problem confronting the beekeeping industry was bee losses due to pesticides—for which there is little disagreement by the beekeeping industry. All indications point to an annual loss by the industry in the neighborhood of 10 % caused by pesticides alone. Few industries can tolerate such losses and survive. The effect of these losses on the adequacy of crop pollination is unknown.

14.12 Indirect Effects of Pesticides on Bees

Besides directly killing the bees, pesticide application produces several indirect effects:

- Reduced foraging due to repellency or due to killing of foragers due to poisoning.
- Sublethal doses can also influence other behaviors such as orientation, dance rhythm, dance velocity, walking speed, and wing beat frequency.
- Physiological injury resulting in reduced longevity.
- Pesticides generally accumulate in combs as a result of absorption from stored pollen and honey/nectar, and may cause chronic paralysis under stress conditions.
- Reduced egg laying and brood rearing due to small doses of pesticides.
- Amorphogenic effects on developing brood and delayed and abnormal development.
- Pesticide application changes the physiology of plants affecting nectar, pollen production, pollen viability, and consequently, bee behavior, nectar pollen collection, honey storage, and pollination.

A schematic model exhibiting direct and indirect effects of pesticides on bee behavior, ecosystem, and crop productivity is given in Fig. 14.2.

14.13 Pesticides Involved: Basic Types and Classes

The following information is presented to help the beekeeper to understand pesticides better and to successfully meet the challenge of pesticides killing honeybees.

14.13.1 *Classes of Pesticides*

The need of human beings to effectively control their environment is most evident in their agricultural pursuits. Modern farming covers large tracts of land under uniform planting, and this has made pest control mandatory. The evolution of pest control

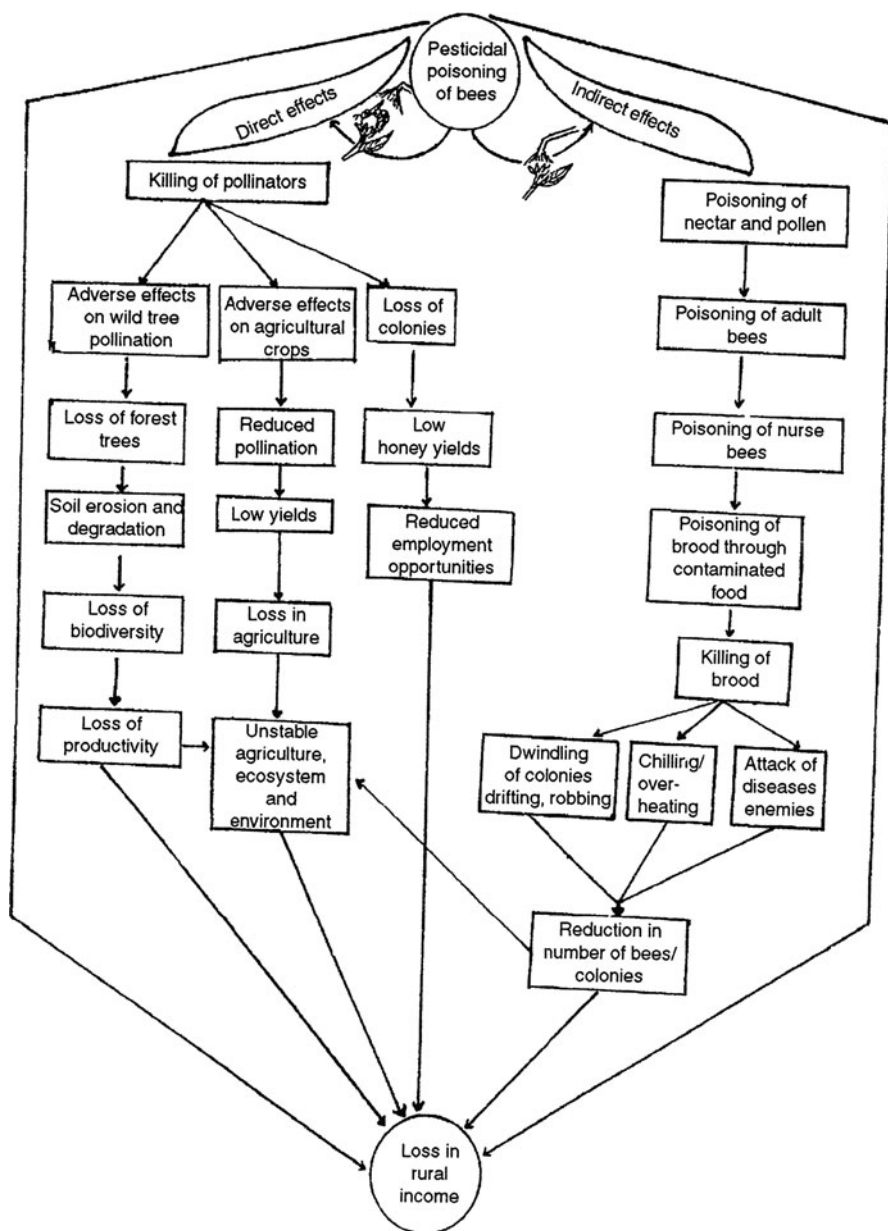


Fig. 14.2 A schematic model exhibiting the impact of pesticides on adult honeybees as well as brood

agents originated with natural products such as arsenicals, petroleum oils, and toxins derived from plants (nicotine and rotenone, for example). The advent of DDT, which was synthesized in a laboratory, heralded an era in which a mature chemical industry would screen synthetic chemicals for pesticidal activity. This effort spawned an

impressive array of insect control agents. The selection of control chemicals is large. However, these materials can be grouped conveniently according to general chemical properties and modes of action.

14.13.1.1 Insecticides

Insecticides affect bees in one or more ways as stomach poisons, as contact materials, and as fumigants. Arsenicals are typical stomach poisons, pyrethrum is a typical contact insecticide, and hydrogen cyanide, paradichlorobenzene, and carbon disulfide are examples of fumigants.

Botanicals

Only a small amount of our insecticides are derived from plants. These sources are cube, derris, nicotine, pyrethrins, ryania, sabadilla, and tephrosia. The bulk of this material is used in households and gardens, and, because of its inaccessibility to bees or the relatively minute amount used, it presents no hazards to pollinating insects. Sabadilla dust is sometimes used on citrus where it can create a bee poisoning problem.

Occasionally, bees are poisoned by feeding on nectar or pollen of certain plants, for example, California buckeye (*Aesculus californica* (Spach) Nutt.), locoweed (*Astragalus* spp.), or mountain laurel (*Kalmia latifolia* L.). Reaction of the bees to these plant poisons can usually be differentiated from those caused by most pesticides.

Inorganics

These pesticides include arsenicals, fluorides, mercury compounds, and sulfur. The method and limited use of the mercury compounds precludes their presenting a hazard to bees. Elemental sulfur alone or when used with other insecticides in the field, presents only a slight repelling action, although fumes from burning sulfur are highly toxic to insects. Fluorides are rarely used on a large scale and present no problem. In certain sections of Europe, fluoride compounds from smelters frequently cause bee damage. Whenever arsenicals are used they pose a serious threat to bees.

Organics

The chlorinated hydrocarbons, organophosphates, and carbamates vary in their toxicity to bees from relatively nonhazardous to highly hazardous, depending upon the individual material or combination of materials.

Chlorinated Hydrocarbons

These include important insecticides such as BHC, toxaphene, and chlordane. The chemicals in this group are slowly reactive chemically, thus persistent in the environment. Biological degradation tends to be slow; hence, storage in fatty and muscle

tissue causes these materials to become concentrated and enter our food chain. The mode of action of chlorinated hydrocarbons is still a subject of active research. They are classified as neuroactive agents which block the transmission of nerve impulses. Specifically, for example, DDT prevents the normal sodium–potassium exchange in the sheath of the nerve fiber, this exchange being the means by which a message is transmitted along the nerve. As chemicals such as DDT are not very chemically reactive, it is felt that the mechanism of reaction with the sheath is not chemical, but rather that the size and shape of a DDT molecule may fortuitously permit it to fit into the proteins of the sheath. Such conceptualizations of the toxicological processes have promoted the search for new chemicals with better toxicological and environmental properties.

Organophosphorus Insecticides

These, today, account for about 30 % of the registered synthetic insecticides/acaricides in the USA. They possess the common characteristic of inhibiting the enzyme cholinesterase, which mediates the transmission of nerve signals. Hence, organophosphates also are neuroactive agents. As their name implies, these materials contain phosphorus, and as a group they include parathion, Systox, DDVP, and malathion. They are quite reactive chemically and are not regarded as persistent in our environment, unless they are microencapsulated.

Carbamate Insecticides

These also are inhibitors of cholinesterase and feature a nitrogen-containing unit known to chemists as a carbamate function. Members of this class of insecticide include carbaryl (Sevin), baygon, Furadan, landrin, and zectran. For the most part, these materials are easily biodegraded and do not constitute the residual hazard of the chlorinated hydrocarbon class of insecticides. Interestingly, cholinesterase inhibition tends to be reversible for mammals and insects alike. A sublethal dose can bring on the usual symptoms of nerve poisoning (tremors, loss of muscular control, incontinence, vomiting), but the poisoned animal will return to normalcy in a very short time.

Pathogens: Bacteria, Protozoans, and Viruses

None of these that are currently recommended or that have been tested for biological control pose a hazard to bees (Cantwell et al. 1972).

Defoliants, Desiccants, and Herbicides

Most tests have shown this class of materials to be nonhazardous to bees, except for their removal of the food source from the plant; however, Morton et al. (1972)

reported that paraquat, MAA, MSMA, DSMA, hexaflurate, and cacodylic acid were extremely toxic when fed to newly emerged worker honeybees at 100 and 1,000 ppm concentrations. Although newly emerged bees do not forage away from the hive, they consume food that others bring in. MSMA, paraquat, and cacodylic acid were also highly toxic when sprayed onto older bees in small cages (Moffett et al. 1972).

Diluents, Synergists, and Activators

There is little information on the influence of these agents on the toxicity of the primary pesticides on honeybees. Possibly different interpretations of the effects of certain pesticides may have been associated with the materials with which they were applied.

Fungicides

As used, the copper compounds, mercury compounds, pentachlorophenol, sulfur, and zineb have caused no trouble to bees. A wide variety of other synthetic chemicals may be applied to crops on which bees may be foraging. Herbicides and fungicides have bases for their activity which render them relatively much less toxic to honeybees. Still such materials are present in the biosphere of the honeybee, and little information is currently available dealing with the effects of these chemicals in combination with insecticides—a situation which occurs often under normal field conditions. Moreover, materials such as herbicides and nonconventional insecticides (such as insect sex attractants and insect growth regulators) to which bees are being increasingly exposed likely will be transferred to honey and stored pollen with, as yet, incompletely documented results.

14.14 Sex Lures, Attractants, and Other Hormones

These usually cause no problems to bees, and their use near bees is generally welcomed. Occasionally, a few honeybees and bumblebees have been found in traps containing Japanese beetle lures (Hamilton et al. 1970).

14.15 Biological Control Agents (Parasitic and Predatory Insects)

Beekeepers would welcome biological control of harmful insects on crops because the control agents likely to be used would prey on the specific insects without harming bees. This would permit bees to forage with safety and effectively pollinate the crop.

14.16 Nonchemical Control

Along with the beneficial aspects of chemical pesticides come problems such as contamination of the environment and killing of beneficial insects, many of which are honeybees. To reduce agriculture's dependency on pesticides, a new concept of pest control has been developed called integrated pest management (IPM). Under IPM, all techniques and methods that are useful in controlling pests are used, including pesticides. However, a farmer applies a chemical pesticide only as a last resort. Primary reliance is on nonchemical controls, such as insect attractants, repellents, traps, insect-resistant plants, insect pathogens (disease), insect predators and parasites, time of planting, cultivation, time of harvest, sterilized insects, quarantines, and other practices. Not all of these techniques are utilized at the same time in controlling a pest; however, all of the control methods are considered noninjurious to honeybees, except for chemical control. Where possible, beekeepers should encourage farmers to use IPM techniques

14.17 Toxicity of a Pesticide

The toxicity of a specific pesticide is a composite of its physical and chemical properties, the method of formulation (description follows), and the inherent ability of the honeybee to deal with the material internally. If the pesticide is of high volatility (an example is the fumigant TEPP), then the chemical may be absorbed through the bee's spiracles or respiratory system. The physical properties of an insecticide and especially of its formulation would be largely responsible for the relative hazard from this mode of entry into the bee. Ingestion of contaminated pollen and nectar offers yet another route of entry. The alimentary tract may become altered or paralyzed, making feeding impossible, or the bee's gut may cease to function. The ability of an insecticide to contaminate nectar and pollen would again be a composite of the physical/chemical properties of the material, its formulation, and the time of application of the spray relative to bloom.

14.18 How Bee Poisoning Occurs

- Bee poisoning generally occurs after a pesticide has been applied to crops or weeds which contain flowers or are providing secretions attractive to bees, e.g., from extra-floral nectaries.
- The pesticide is applied directly onto bees foraging on the crop.
- Bees fly to the treated plants and collect contaminated nectar and/or pollen.
- Bees collect contaminated water on or near treated plants.
- Bees forage on a cover crop associated with the treated crop, e.g., clover in an orchard.

- Pollen collecting bees collect pesticide dust and/or contaminated pollen and return to the hive.
- Pesticides drift from their point of application onto flowering plants or across apiaries.

14.19 Symptoms of Bee Poisoning

Bees come in contact with pesticides during foraging. There can be stomach poisoning to bees and also to brood when fed on contaminated pollen. Some pesticides may even cause hazards by fumigant action. After gaining entry into the body, different pesticides having different modes of action. Some of the most important and common symptoms of pesticides poisoning in bees are discussed further.

Presence of large number of dead bees in front of bee hive. Individual bees that have been poisoned frequently are seen crawling on the ground near the entrance or twirling on their side in a tight circle. Others appear to be weak or paralyzed. These gross symptoms of poisoning vary with the type of pesticide and the degree of exposure. Foraging bees also may die in the field or on the flight back to the hive. Bees become paralytic, lose the power of orientation; legs, wings and digestive tract stop functioning and poisoned bees show uncoordinated movements. Abdomen becomes distended. (Table 14.1).

Dead adult bees typically, but not always, have their wings unhooked and at odd angles to their body, their proboscis fully extended, and their hind pair of legs outstretched behind them.

Workers can get confused, forget their path, and could not reach back to hives, hence their number is reduced. Bees may perform abnormal communication dances on the horizontal landing board at the hive entrance while under the influence of insecticide poisoning. Disorganized behavior patterns may lead to lack of recognition of affected field bees by guard bees which may harm the affected bees.

Regurgitation of contents of gut is noticed particularly in case of organophosphate insecticidal poisoning. Stupor, paralysis, and abnormal activities of bees are commonly caused by chlorinated hydrocarbons and organophosphorus insecticides.

Guard bees are also confused due to toxic effect of pesticides. Brood chilling can occur due to reduction in the population of adult bees. Dead brood can be seen inside the colonies if poisoned pollens are stored and fed to the brood.

Sometimes, the queens are also affected. Queens may stop laying eggs or lay eggs in irregular pattern, there may be brood in only some of the cells of the brood area as in case of the colonies suffering from foulbrood. Sometimes queenlessness may develop. Queen cells may be raised and queens may be superseded in colonies which survive.

If gamma-BHC (lindane) and endosulfan etc. from organochlorine group are used on crops, the affected bees cannot move properly and their legs are adversely affected. They appear as though they had been chilled. Most of such bees die away from their hives.

Table 14.1 Comparative symptoms in honeybees poisoned by toxic chemicals and selected plants. (Modified from Burnside and Vansell 1936)

Source of poison	Stages most affected	Effect on adult	Effect on brood	Effect on colony
Toxic chemicals	Adult	Field bees die in or near hive; nurse bees may also die; queens usually not affected	Usually few larvae killed; symptoms of starvation may be evident if adult population reduced severely	Weakened or killed; many dead bees near hive
California buckeye (<i>Aesculus californica</i>)	Young brood	Emerging young workers often deformed, pale; some hairless and tremble; queens lay eggs at reduced rate, cease, or become drone layers	Eggs normal at outset, later fail to hatch or all are drone eggs; larvae die soon after hatching and disappear; little or no capped brood, if present, scattered	Weakened or killed; may be many dead bees near entrance; supersedure of queen may fail
Yellow jessamine (<i>Gelsemium sempervirens</i>)	Larva, pupa, and young adult	Young workers affected and soon die; old adults appear normal	Pupae die in cells and become mummified	Slightly to severely weakened
Loco plants (<i>Astragalus</i> spp.)	Adult and pupa	Field bees die; some become black and tremble; queen may die	Many cells contain dried pupae	Population dwindles; colony may die
False hellebore (<i>Veratrum californicum</i>)	Adult	Many field bees die between plants and hive; adults die in curled state; queens not affected	No effect	Field population lost
Southern leatherwood (<i>Cyrilla racemiflora</i>)	Larva	No effect	Many blue or purple larvae die in cells when nearly mature	Slight to severe weakening

If malathion, dimethoate, and other related insecticides from organophosphate group are used, a watery liquid substance comes out from their mouth due to which the whole body of bees becomes wet, swelling of abdomen takes place, and the pair of wings stick to the body of bees. The behavior of such bees also changes and they die near their hives.

If insecticides of carbamate group such as carbaryl, carbofuran, etc. are used for insect pest control, the affected bees become more angry and are not able to fly properly. Most of the affected bees die near their hives. In such a bee colony, the queen stops laying eggs. In extreme cases of pesticide exposure, the house bees also die. When the house bees die, the brood will show signs of neglect or poisoning and many, or all, immature bees still in the cells may die. Some pesticides, particularly systemic pesticides, have a less noticeable, but debilitating effect, resulting in an overall weakening of the colony. Signs are reduction in adult bee numbers and stages of the brood cycle or complete brood cycles missing. In severe cases, when insufficient numbers of adult bees remain, temperature and humidity control in the brood area is lost and brood is not fed. Brood die from chilling, overheating, or starvation.

By observing symptoms one can find out the group of insecticides sprayed on the crop in the vicinity of the apiary. In addition to this a scientist or a beekeeper as well as farmers should also know the degree of toxicity of different insecticides to the bees so that a person can spray such an insecticide which is quite effective against pests but least toxic to the bees.

14.20 Groups of Insecticides Based on Their Toxicity to Bees

Toxicity is life property of a substance (insecticides) that causes any adverse effect in an organism. The toxicity may be due to single short-term exposure period (acute toxicity) or due to repeated/continuous exposure within less than half the lifetime of that animal (subacute toxicity) or repeated exposure over a period of at least half the lifetime of that organism (chronic toxicity). On the basis of toxicity to bees, the insecticides can be grouped into the following three categories:

14.20.1 More Toxic Insecticides

The insecticides which have adverse effect on bees even after 90 h (about 4 days) comes under this category. Such insecticides are carbofuran, dichlorovous fen-thion, monocrotophos, fenitrothion, lindane, malathion, carbaryl, methl-parathion, chlorpyriphos, dimethoate, phosphamidon, diaginon, etc.

14.20.2 Less Toxic Insecticides

Those insecticides whose residues are less persistent in nature and remain toxic to bees upto 90 h (less than 4 days) fall in this category. Such insecticides are endosulfan and phosalone. Besides these, all systemic insecticides also fall in this category if used properly. These insecticides should be sprayed after 3 p.m. and bees should be allowed to go a little late next morning in the fields on crop plants so that by the time

the bees reach the fields, most of the toxicant enters inside the plant system being systemic in nature, resulting in minimum harm to the bees.

14.20.3 Nontoxic Insecticides

There are some chemicals which are very effective for control of insect pests but nontoxic to bees. They are some bacteria (B.T. formulations), viruses (NPV) and insect growth regulators, etc. Besides these, fungicides, weedicides, and some plant growth regulators have also been found safe to bees.

14.21 Relative Toxicity of Pesticides

The kill of bees can be classified as:

< 100 per day	Normal die off rate
200–400 per day	Low kill
500–900 per day	Moderate kill
> 1,000 per day	High kill

14.22 The Sublethal Effects of Pesticides on the Behavior of Bees

The effects of pesticides on nontarget organisms have been studied extensively. Despite research data indicating the severe mortality rate on insects, less attention has been paid to the sublethal effects. Application of insecticides is often allowed during the flowering period of a given crop. However, even when insecticides are not sprayed on flowers, the residues of the compounds still contaminate nectar and pollen in sublethal doses via both active and passive transport (Thompson 2001). Many insecticides have been described as safe to bees because they do not kill them, although sublethal doses may result in a decrease in their foraging and navigational abilities (Gels et al. 2002). Under certain circumstances, the sublethal effects may cause more harm than lethal doses since they affect the survival of the brood and colony. In the colonies of social insects the division of labor plays an important role. Each worker has specific, often age-dependent tasks. Treatment of honeybees with juvenile hormone analogs (synthetic hormone-like compounds used as insecticides) results in a decreasing ability of young emerging bees to feed larvae, due to the early degeneration of the hypopharyngeal glands and precocious foraging ability (Tasei 2001).

Changes in the division of labor of honeybees—decreased house cleaning abilities, delayed onset and duration of foraging and handling of nectar—have also been recorded (reviewed by Thompson 2003). Organophosphate insecticides may

decrease the longevity of honeybees (Johansen and Mayer 1990). Juvenile hormone analogs also affect the overwintering of colonies (Thompson et al. 2005).

Foraging depends on the bee's ability to discriminate odors, to learn, to communicate, and to orientate to its environment; altering these systems may result in a decrease in foraging. The bees' orientation and communication ability have been found to be affected by sublethal doses of organophosphorus (Schricker and Stephen 1970), synthetic pyrethroids (Cox and Wilson 1984; Vandame et al. 1995) and at least one neonicotinoid (Bortolotti et al. 2003). Pyrethroids and neonicotinoids have also been shown to affect both foraging activity (Thompson 2003) and learning capacities (Decourtye et al. 1999, 2003; Guez et al. 2001; Ramirez-Romero et al. 2005). Pyrethroids may also affect thermoregulation (Jagers op Akkerhuis et al. 1999b; Belzunces et al. 2001) in cooler climates, that can lead to decreased flying ability. The decrease in foraging and in returning of foragers reduces the brood production (Thompson 2003), and weakens a colony's potential for surviving the winter.

The reduction of the brood may have more damaging consequences for honeybees than simply the moderate loss of foragers (Haynes 1988). Apart from brood mortality there can be changes in larval development (both prolonged development time and malformations may occur) due to the contamination of the food by pesticides (Tasei 2001). Some organophosphates have affected the queen's status or have interfered with a colony's ability to requeen itself (Stoner et al. 1985; Thompson et al. 2005). In solitary bees, pyrethroids have been found to affect the queen's fecundity (Tasei et al. 1988). Neonicotinoids (Tasei et al. 2000) and organophosphates (Johansen and Mayer 1990) have decreased the bumblebees' brood production.

In addition to ignoring the sublethal effects of insecticides, there exists the problem of extrapolating data from honeybees to bumblebees. Pesticide risk assessments for honeybees are based on hazard ratios which rely on application rates and toxicity data that are unlikely to be appropriate for bumblebees. Bumblebees are active at different times and on different crop species and, therefore, are likely to have different exposure profiles. Unlike honeybees, deaths of bumblebees due to pesticides are unlikely to be reported, since the bees are not kept domestically and die in small numbers (Thompson and Hunt 1999).

14.22.1 Poisoning and Developmental Stages

Worker bees are those primarily affected by pesticides. The symptoms of poisoning can vary depending on the developmental stage of the individual bee and kind of chemical employed.

14.22.2 Development of Adult

It takes worker bees about 21 days to develop from egg to adult. During this process, each individual passes through a larval (feeding) stage followed by a pupal (transformation) stage. The larval stage is the most susceptible to pesticide poisoning during development.

14.22.3 *House Bees*

These bees are emerged worker adults up to 21 days of age. They care for the brood, process pollen and nectar gathered in the field by older workers, and clean the nest. Eventually, they too will become field bees. House bees are usually poisoned by contaminated pollen which is collected in the field, brought back, and stored in the hive. As house bees are killed, there are fewer bees to tend the brood and further decline in population results.

14.22.4 *Field Bees*

These bees are workers 21 to approximately 42 days of age. There appears to be no greater risk in bee society than to be a field bee. Should the insect avoid all the potential pitfalls due to predators like spiders, toads, or skunks, it is still vulnerable at all times to the numerous pesticides applied in commercial agriculture, mosquito control, and home gardens. Most times, field bees are killed by contact with pesticides in the field, but other times they collect contaminated nectar and pollen and contribute to poisoning their sisters in the colony. If field bees are killed, then young bees are forced into the field earlier than normal, disrupting and thus disorienting the colony.

While foraging, field bees may range as far as 2–5 miles from a colony. They usually seek nectar and pollen systematically, not randomly, and once a food source is found, bees prefer to work that particular source to exhaustion before changing plants. This kind of resource partitioning by bee colonies accounts for the inconsistency observed many times between colonies undergoing pesticide poisoning in the same location. The bees are not all working the same plants and so some are affected more than others. Often it is those bees with established flight patterns located in an area before a pesticide is applied that are most damaged. Those placed in a field immediately after application are less affected by the pesticide because it takes some time for the bees to scout an area and locate food sources.

On the basis of mode of action, the insecticides are classified as given in Table 14.2.

The relative toxicity of different insecticides to honeybees has been given in tables 14.1, 14.2, 14.3, 14.4, 14.5, 14.6, 14.7, 14.8, 14.9 and 14.10 and also as follows:

LD ₅₀ (μg/bee)	Classification
> 100	Virtually nontoxic
11–100	Slightly toxic
2–10.99	Moderately toxic
< 2.0 (i.e., 0.001–1.99 μg/bee)	Highly toxic

Table 14.2 Classification of insecticides on the basis of their mode of action

Group	Subgroup	Example
Physical poisons	–	Heavy mineral oils, inert dusts
Protoplasmic poisons	–	Heavy metals, e.g., Hg, acids
Metabolic inhibitors	Respiratory poisons	HCN, CO, H ₂ S, rotenone, dinitrophenols
	Inhibitors of mixed-function oxidase	Pyrethrin synergists
	Inhibitors of carbohydrate metabolism	Sodium fluoroacetate
	Inhibitors of amine metabolism	Chlordimeform
	Insect hormones	Juvenile hormone analogs
	Inhibitors of chitin synthesis	Diflubenzuron
Neuroactive agents (nonmetabolic)	Anticholinesterases	Organophosphorus compounds, carbamates
	Effects on ion permeability	DDT analogs, pyrethroid
	Agents for nerve receptors	Ach*nicotinoids, Nereistoxin analogues, GABA*cyclodienes, HCH, Avermectins (Spinosoids) Octopamine*Formamidines
Hormone mimics	–	Methoprene
Stomach poisons	–	<i>Bacillus thuringiensis</i> toxin, Thiodicarb

Table 14.3 Pesticide risk to bees

Risk rating	Chemicals	Remarks
High risk to bees foraging even 10 h after spraying	Carbaryl, chlorpyrifos, diazinon, dimethoate, omethoate, methomyl, fenthion, methamidophos, methidathion, monochrotophos	These should never be sprayed on flowering crops especially if bees are active and the crop requires pollination
Moderate risk with some losses expected 10 h after spraying	Acephate, demeton-s-methyl	
Some risk with low chance of losing bees 3–5 h after spraying	Endosulfan, dicofol, pirimicarb, petroleum oils, most pyrethroid chemicals, trichlorfon	There is little risk of losing bees if these chemicals are sprayed in the evening when foraging has ceased
No risk even if sprayed over foraging bees	<i>Bacillus thuringiensis</i> , propargite, oxythioquinox	

Table 14.4 Pesticides most toxic to bees (LD_{50} 0.001–1.99 $\mu\text{g}/\text{bee}$). Application of these pesticides on blooming crops or weeds may cause severe damage to bees and may be toxic even after 10 h of spraying

Acephate fenthion ^a
Aldicarb G (applied 4 weeks before bloom) heptachlor ^a
Aldrin ^a isobenzan
Aminocarb lean arsenate
Azinphosethyl malathion D ^a
Azinphosmethyl malathion ULV
Benzene hexachloride ^a methamidophos
Bomyl methidathion
Calcium arsenate methiocarb
Carbanolate methomyl D
Carbaryl ULV (over 0.4 kg/ha) methyl-carbophenothion
Carbofuran F methyl parathion ^a
Carbophenothion D mevinphos
Chlorpyrifos ^a mexacarbate
Chlorthion monocrotophos
Colep Naled D
Crotoxyphos omethate
Diazinon ^a paraoxon
Dicaphon parathion ^a
Dichlorvos ^a permethrin
Dicrotophos phethoate
Dieldrin phosmet
Dimethoate ^a phosphamidon ^a
Dinitroresol phoxim
Dinoseb propoxur
EPN pydrin (over 0.1 kg/ha)
Ethyl-methyl guthion pyramat
Famphur resmethrin
Fenamiphos sulfotep (p)
Fenitrothion ^a sulprofos
Fensulfothion thionazin

For minimal hazard to honeybees, the insecticides listed should NOT be applied on blooming crops or weeds. Their residual toxicity is usually high even after 10 h

^aThere is an Indian Standard for one or more formulations of these pesticides (India Standards Institution 1979)

Table 14.5 Pesticides very toxic to bees

Malathion EC ^a
Naled WP
Phorate EC
Pydrin (0.1 kg/ha or less)

For minimal hazard to honeybees, the insecticides listed should be applied only during late evening. Their residual toxicity is usually low within 8 h

^aThere is an Indian Standard for one or more formulations of this pesticide (Indian Standards Institution 1979)

Table 14.6 Pesticides highly toxic to bees

Amidithion Kroneton
Aramite D larvin
Binapacryl ^a Leptophos
Carbaryl ULV (0.5 kg/ha or less) lethane 384 special
Carbophenothion malathion MA
Chlordane ^a malonoben
Chlorfenvinphos menazon
Chlorpyrifos or chlorpyrifos MA methomyl S, SP
Coumaphos methoxychlor
DDD methyl-demeton
DDT ^a Naled EC
Demeton nissol
Dichlorfention or dichlorfenthion oil sprays (superior type)
Dichlorvos MA oxamyl
Dieldrin G oxydemeton-methyl ^a
Dilan phorate G
Dimetilan phosalone ^a
Dinobuton phostex
Dioxathion pirimicarb
Disulfoton EC propoxur MA
Endosulfan ^a Propyl thiopyrophosphate
Endrin ^a rotenone D
Ethion sabadilla
^a Ethyl-DDD solvigran
Fenchlorphos strobane
Fenthion G or MA temephos ^a
Fonofos TEPP
Formetanate tetrachlorvinphos
Garrathion tetram
Heptachlor G thanite (isobornyl thiocyanate)
Isobornyl thiocyanate thioquinox
Isodrin toxaphene ^a
Isolan tranid
Isopropyl parathion trichlorfon ^a
Malathion EC ^a
Naled WP
Phorate EC
Pydrin (0.1 kg/ha or less)

For minimal hazard to honeybees, the insecticides below should be applied only during late evening. Their residual toxicity is usually low within 8 h

^aThere is an Indian Standard for one or more formulations of this pesticide (Indian Standards Institution 1979)

Table 14.7 Pesticides least toxic to bees (LD₅₀ 2.0–10 µg/bee)

Allethrin fenazaflor
Amitraz fenbutatin-oxide
Bromopropylate fenson
Butoxy thiocyanodiethyl ether fensulfothion G
Carbaryl G genite 923 or genitol 923
Carbofuran G hydroprene
Chlorbenside lime-sulphur or lime-sulfur ^a
Chlordecone malathion G
Chlordimeform mirex G
Chlorfenethol nicotine sulphate
Chlorfenson oxythane
Chlorfensulphide propargite
Chlorobenzilate propoxur G
Chloropropylate pyrethrum ^a
Cryolite quinomethionate
Cyhexatin rotenone EC
Dicofol ^a ryania
Dienochlor schradan
Diffubenzuron sodium fluosilicate baits
Dikar sulphenone
Dinex sulphur or sulfur ^a
Dinocap tetradifon
Disulfoton G

The insecticides below can be applied at any time with reasonable safety to honeybees. Their toxicity is usually low with direct application. The following pesticides should be applied in the late evening when bees are not foraging in the field. Bee hives should not be directly exposed to these insecticides. For minimal hazard to bees, dose, the timing, and methods of application are very important.

^aThere is an Indian Standard for one or more formulations of this pesticide (Indian Standards Institution 1979)

Table 14.8 Nontoxic pesticides (LD₅₀ above 11 µg/bee)*A. Insecticides and acaricides*

Acaraben (chloro-benzilate)	Ethodan	Omite
	Fundal	OMPA (schradan)
Allethrin	Galecron (chlorophenamidine)	Ovotran (ovey)
Aramite		Phostex
Bacillus thuringiensis	Heliothis virus	Phrethrin
	Kelthane (dicofol)	Rhothane (TDC)
Cryolite	Kepone	Rotenone
Delnav (dioxathion)	methoxychlor	Ryania
Dessin	Mitox (chlorbenside)	Sabadilla
Dilan	Morestan	Sulphenone
Dylox (trichlorfon)	Nemagon	Tedion (tetradifon)
Eradex	Neotran nicotine	Toxaphene

Table 14.8 (continued)*A. Insecticides and acaricides**B. Fungicides*

Arasan (thiram)	Cyprex (dodine)	Manzate (maneb)
Benlate (benomyl)	Dexon	Mylone
Bordeaux mixture	Dichlone	Parzate (nabam)
Copper oxychloride sulfate	Difolatan	Phaltan (folpet)
	Dithane M-45	Polyram
Copper sulfate (monahydrate)	(Folcid)	Sulfur
	Glyoxide (glyodin)	Thynon (dithianon)
Cuprous oxide	Karathane (dinocap)	Zerlate (ziram)

C. Herbicides

Amitrol	Eptam (EPTC)	Picloram
Ammate (ammonium sulfamate)	Folex (merphos)	Planavin
	Herbisan (EXD)	Princep (simazine)
Atrazine	Hyvar (bromacil)	Randox (CDAA)
Banvel (dicamba)	Igran (terbutryne)	Sinbar (terbacil)
Betanal (phenmedipham)	IPC	Stem F-34 (propanil)
	Karmex (diuron)	
Caparol (promytryne)	MCPA	TOK (nitrofen)
Casoron (dichlobenil)	Milogard (propazine)	Trysben (2, 3, 6-TBA)
	Monuron	Vege dex (CDEX)
Dalapon	NPA	2, 4-D
DEF	Paraquat	2, 4-DB
Diquat		2, 4, 5-T

These can be used around bees with minimum injury. They should be applied during late evening, night, or early morning. These products can be used on fields or near hives with minimum damage to bees; in fact, a few of the listed acaricides can be used to control bee mites within the hive

Table 14.9 Common insecticides and miticides and their relative risk to honeybees (Modified from Johansen and Mayer 1990; Delaplane 1993)

Active ingredient	Trade names	Risk class ^a	LD ₅₀ ^b	Residual ^c
Acephate	Orthene	I	1.2	1 to > 3 days
Aldicarb	Temik	I	0.35	> 1 to > 2 days
Azinphos methyl	Guthion	I	0.43	2 to > 5 days
Bacillus thuringiensis	Biobit, Cutlass, Dipel, Javelin, Thuricide	III	NA	< 2 h
Carbaryl	Sevin	I	1.5–26.5	< 2 h to 12 days
Chlorpyrifos	Dursban, Lorsban	I	0.11	5 h to 6 days
Cyhexatin	Plictran	III	NA	< 2 h
Cypermethrin	Ammo, Cymbush	III	NA	< 2 h to > 3 days
Diazinon	Diazinon	I	0.37	< 1 to 2 days
Dicofol	Kelthane	III	NA	< 2 h
Dicrotophos	Bidrin	I	0.3	1 day to 1 days

Table 14.9 (continued)

Active ingredient	Trade names	Risk class ^a	LD ₅₀ ^b	Residual ^c
Diflubenzuron	Dimilin	III	NA	< 2 to 6 h
Dimethoate	Cygon, Defend, Rebelate	I	0.19	< 2 h to > 3 days
Disulfoton	Di-syston	II	6.12	< 2 h to 7 h
Endosulfan	Thiodan	II	7.8	< 2 h to 1 day
Ethion	Ethion oil	III	NA	< 2 h
Fluvalinate	Mavrik	III	65.8	< 2 h
Fonofos	Fonofos Dyfonate	II	8.68	< 2 to 6 h
Formetanate hydrochloride	Carzol	II	9.2	< 2 h to 2 h
Lindane	Lindane	I	NA	> 2 days
Malathion	Cythion, Malathion	I	0.73	< 2 h to 5 days
Methamidophos	Monitor	I	1.37	4 h to 1 day
Methidathion	Supracide	I	0.24	1 to 3 days
Methomyl	Lannate	I	1.29	< 2 h to > 1 day
Methoxychlor	Marlate, Methoxychlor	III	NA	< 2 h
Methyl parathion	Pennacp-M	I	0.11–0.24	< 1 to > 7 days
Mevinphos	Phosdrin	I	0.3	< 2 to < 5 h
Naled	Dibrom	I	0.49	2 h to > 1 day
Oxamyl	Hydrate	II	10.3	< 2 to 12 h
Oxythioquinox	Morestan	III	NA	< 2 h
Parathion	Parathion	I	0.18	10 h to > 1 day
Permethrin	Ambush, Permethrin, Pounce	I	0.16 12	< 2 h to > 3 days
Phorate	Thimet	II	10.25	< 2 to 5 h
Phosmet	Imidan	I	1.13	8 h to > 3 days
Profenofos	Curacron	II	3.46	< 2 to 9 h
Propargite	Omite, ornamite	III	NA	< 2 h
Sulprofos	Bolstar	II	7.22	< 2 h to > 1 day
Thiodicarb	Larvin	II	7.08	< 2 h
Trichlorfon	Dylox, Proxol	III	NA	< 2 to 6 h

Never spray during bloom periods unless it is absolutely necessary. If treatment is unavoidable, choose a product with a high LD₅₀ and short residual. If a more toxic chemical is required, choose a residual under 8 h and spray at night

^aRisk classes: I highly toxic to honeybees, II moderately toxic to honeybees, III relatively nontoxic to honeybees. The risk class is closely associated with the LD₅₀

^bLD₅₀ = the Lethal Dose required to kill 50 % of the test honeybees, expressed in micrograms per bee. The smaller the LD₅₀, the more toxic the product

^cPeriod of residual toxicity to honeybees after application. Evening applications of products with residuals of 8 h or less generally cause only moderate harm to bees, even if the LD₅₀ is small. For example, mevinphos is very toxic to bees, but because it has a short residual, it is fairly safe for early evening applications

Table 14.10 Pesticides recommended as relatively safe for honeybees (Indian Standards Institution 1979)

Pesticide	Use class	Cotton	Maize	Brassicas	Vegetables	Fruit	Others
Amidithion	3						X
Carbophenothion	3						X
DDT	3	X			X	X	X
Demeton methyl	3	X	X	X	X	X	X
Disulfoton	3, 4						X
Endosulfan	3	X	X		X		X
Endrin	3	X				X	X
Ethion	3						X
Fenchlorphos	3						X
Isolan	3						X
Menazon	3			X	X	X	X
Methoxychlor	3						X
Naled	2, 3						X
Phorate	2, 3						X
Ryania	4						X
Temephos	3						X
Toxaphene	3		X		X	X	X
Trichlorfon	3		X				X

The recommendations apply to the crop specified, and for time and method of application stated in the publication

14.23 Factors Influencing the Toxicity of Insecticides to Bees

14.23.1 Temperature

Temperature is probably the most significant factor causing differences in the toxicity of pesticides. Immediate effects may be much greater at higher temperatures whereas, residual effects are likely to be less because the toxic materials break down more quickly.

14.23.2 Age and Size of the Bees

From the general principles, body size is likely to have a direct effect on the susceptibility to the contact pesticides. Smaller bees have a higher surface to volume ratio and hence, contact poison will be more toxic to them than larger bees.

14.24 Protection of Bees from Pesticide Poisoning

Several management practices are known that may help protect bees from pesticide-caused mortality.

14.24.1 Detoxification of Insecticides by Metabolic Enzymes

The adverse effects usually induced by pesticides are limited by the action of a large set of metabolic enzymes. Although honeybees have fewer genes involved in detoxification than other insects (Claudianos et al. 2006), they are not necessarily more sensitive to pesticides (Hardstone and Scott 2010). In honeybees, detoxification processes occur mainly in both midgut and fat body (Robinson and Nation 1984), very similar to those of mammals (Terriere 1984). Induction of microsomal monooxygenases and glutathione-S-transferase is one of the key mechanisms of insect sensitivity to pesticides (Terriere 1984; Johnson et al. 2006). The role of detoxification enzymes, however, is not limited to the protection of insect against the deleterious effects of pesticides. These enzymes are also involved in the metabolism of endogenous compounds such as hormones and pheromones (Claudianos et al. 2006). Therefore, changes in the activity of the detoxification system can lead to variations in honeybee sensitivity to pesticides and more generally to alteration of their physiological homeostasis.

Stenerson (1965) first speculated on the role of bacteria-influenced metabolism of DDT on resistant mosquitoes. Sharma and Nath (1996) showed adaptation of carbaryl of bacterial isolates from *Apis cerana* bees. Treatment of bees with these modified isolates resulted in decontamination of bees and enhanced tolerance to carbaryl. Sharma and Nath (2000) found that all the bacterial isolates obtained from *Apis cerana* (*Citrobacter* sp., *Enterobacter aerogenes* and unidentified sp.) have the ability to grow and degrade carbaryl in nutrient broth and basal medium salts.

14.24.2 Precautions

Do not apply any insecticide unless the crop is so heavily infested and the insect in question has reached the economic threshold value (ET-value). If necessary, then

1. Read the pesticide label carefully: Read the pesticide label. Pesticides and formulations which pose a special hazard to bees are required to include a notification on the label.
2. Use less toxic compounds: Choose an insecticide of low toxicity to bees that will provide the needed pest control.
3. Use less toxic formulations: Mostly dust, wettable/water dispersible liquid, and granular formulations of insecticides are used to control insect/pests of various crops. Select the safest formulation of the pesticide that is available for the intended use. "Drifting" of the pesticide from the target pest and/or crop to areas frequented by bees should be minimized.

Types of formulation and their toxicity to bees

- a. Dust formulations: These formulations if used on crop result in maximum toxicity to bees and other pollinators as compared to other formulation. Dusts almost always drift more than other formulations and are generally more dangerous to bees than are sprays or granular application.

- b. W.P./W.D.P. formulations: These formulations are less toxic to bees as compared to dust formulations but definitely more toxic in comparison to other formulations of insecticides.
 - c. Liquid formulations: Of course liquid formulations are less toxic to bees as compared to dust and wettable or water dispersible powder formulations but they are more toxic as compared to granular formulations.
 - d. Granular formulations: Among the insecticidal formulations which are commonly used to control pests of various crops, it is the granular formulation which is least toxic to honeybees visiting flowers to collect nectar and pollen. Further, ground applications are always safer than aerial applications.
 - e. Microencapsulated pesticides: Microencapsulated pesticides present a very distinct and serious threat to honeybees. The particle size of this formulation is very similar to that of pollen, and adult honeybees may carry this pesticide back to the hive, where it will be combined with pollen that is being stored in the hive. This pesticide will not kill the adult bees that collected it, but it will kill the immature stages of the bees and the young adult nurse bees that feed the brood. Bees have little protection from this product.
4. The mode of pesticide application: The mode of pesticide application is also important, particularly when it comes to drift. Aerial applications are generally more dangerous than applications by ground equipment, because of the location of target pests and/or crops near foraging bees or beehives. Never apply a pesticide directly over beehive.
 5. Do not treat crops in bloom: Whenever possible, do not treat crops in bloom. If treatments are needed during bloom, choose a short-residual material. Make applications during the evening, when bees are not actively foraging. Honeybees are active primarily during the morning and early afternoon. Many pesticides can be effectively applied in the late afternoon or evening with relative safety to bees. Remove weed blooms in orchard groundcover and in field edges before spraying. Flowering weeds may be removed by mowing or with a herbicide.
 6. Minimize spray drift: Minimize spray drift onto adjacent crops or other plants in bloom. Honeybee hives should not be placed next to fields or orchards that are likely to be treated with pesticides toxic to bees. A small number of hives may be protected from pesticides by covering the colonies with wet burlap for a period of 1–2 days. In some cases, it may be practical to move hives to a less exposed site. Beekeepers should inform farmers of the location of hives.
 7. Use pesticides only when needed: Foraging honeybees, other pollinators, and insect predators are natural resources and their intrinsic value must be taken into consideration. Vegetable, fruit, and seed crop yields in nearby fields can be adversely affected by reducing the population of pollinating insects and beneficial insect predators. It is always a good idea to check the field to be treated for populations of both harmful and beneficial insects.
 8. Apply pesticide when bees are not flying: Bees fly when the air temperature is above 55–60 °F and are most active from 8 a.m. to 5 p.m. Always check a field for bee activity immediately before application. Pesticides hazardous to honeybees must be applied to blooming plants when bees are not working, preferably in the

early evening. Evening application allows time for these chemicals to partially or totally decompose during the night.

9. Do not contaminate water: Bees require water to cool the hive and feed the brood. Never contaminate standing water with pesticides or drain spray tank contents onto the ground, creating puddles.
10. Identify attractive blooms: Bees also visit flowers of some weeds; hence before spraying, remove such weeds from fields which might attract bees. In many instances bees have been killed even though the crop being sprayed was not in bloom. Many times these attractive blooms can be mowed or otherwise removed, although mowing can result in destroying other beneficial insect habitat or force destructive insects into the crop being cultivated.
11. Disposing of unused pesticides: Follow proper precautions in disposing of unused pesticides and pesticide containers. Be particularly careful not to contaminate water with pesticides, as that water may be collected by bees which would result in bee kills. Pesticide dusts and small granules should not be left open or carelessly thrown anywhere because bees are likely to collect such dusts during acute pollen dearth period.
12. Use IPM to reduce pesticide hazard. IPM is an important approach to controlling insects, mites, weeds, and diseases of crops. Whenever possible, it is wise to use such IPM practices viz., recognition of existing biological controls and evaluating their value before applying any pesticides, using pesticides that are less harmful to beneficial organisms, and timing pesticide applications so they are less harmful to beneficial organisms. Biological control of organisms may effectively control pests even though they are not recognized. Even pests that occur regularly, such as spider mites on field corn or greenbugs on sorghum, may be effectively controlled under some conditions. Before applying pesticides, fields should be examined for the activity of natural enemies. If large numbers are present, pesticide use may be deferred or avoided.
13. Use of repellents: Chemical repellents have been studied for many years. The repellent is added to the pesticide before field application and is intended to discourage bees from visiting plants until the pesticide becomes relatively nontoxic. Field tests showed several compounds to have repellency, but more research is needed before they are used commercially by farmers.
14. Notify beekeepers and inform presence of apiary: If your bees are located in any area where pesticides are commonly used, then identify yourself as a beekeeper to any neighbors who may use pesticides. Explain to growers the importance of your honeybees in the pollination of crops being grown in nearby fields so that they may consider the value of bees in pollination before applying any pesticides that may kill the pollinating insects. Do not place apiaries in areas that are used to grow crops that require heavy and frequent usage of pesticides. Notify beekeepers who have beehives near an area to be treated with a pesticide so that they may attempt to protect their bees. If bee boxes are kept near or in the crop, take them away or cover them with jute bags at the time of insecticidal spraying. In the areas of bee-keeping, there should be good coordination between the bee-keepers and crop/fruit vegetable growers so that the farmers may inform

the beekeepers well in advance so that either they remove the colonies or keep the bees confined inside the hive during the application. While confining the bees, due attention should be paid to the following points: (a) Proper space: Sufficient and proper space for all the bees including foragers should be made available. (b) Proper ventilation: Proper ventilation must be provided at the top or sides of the hive, not only through the entrance, because this may get blocked by dead bees. Ventilation screen should have as large a mesh as possible (c) Shade: Shade is usually provided by the use of local materials. It must not hinder the flow of air past the hives. (d) Covering hives: Covering the hives with wet clothes or gunny bags is very useful because evaporation of water helps to reduce the rise in colony temperature. If pesticides are applied aerially, then cover whole of the hive. (e) Water availability: Provide water inside the hives for bees. The bees take it and spread it out in the hive where it evaporates and thus reduces the temperature. (f) Minimize the period of confinement: The confinement must of course continue as long as the pesticide near the hive retains unacceptable toxicity and its duration can satisfactorily be reduced by application of less persistent insecticide. If there is no store in the hive, pollen supplement and sugar syrup may also be provided. In case of adverse effect on bees including brood due to pesticidal contamination of pollens, beekeepers can overcome this difficulty by prevention of pollen being stored in the hive and provision of a safe pollen supply inside the hive. Provision of pollen cakes in the hive during this period greatly reduces the collection of toxic pollen. Cake of pure pollen were more effective than pollen supplement made with soybean flour.

14.25 Management of Poisoned Colonies

Shift colonies away from foraging range from the source of poisoning. Keep bees warm by removing excess supers.

If a pesticide carried by the bees infiltrates the combs and contaminates the nectar or pollen, or both, then the combs may need to be replaced before a colony will recover. Remove contaminated pollen stored in combs by dipping the combs in water and washing by slight shaking or the entire comb melted and replaced with wax foundation. Before washing or melting combs, however, a sample of the pollen, wax, and honey should be analyzed chemically to determine the amount of pesticide residue present, if any.

Provide sugar syrup and pollen substitute. Feed colonies inside the hive with a 1:1 water:sugar syrup until recovery. Loss of field bees results in a lack of fresh nectar and water being brought into the hive. Add frames of sealed brood and adult bees from healthy hives, if required. Moreover, colonies weakened by insecticide treatment should be fed sugar syrup and pollen cake to stimulate brood production as an aid to population recovery. Frequently, weak colonies must be united to save the remaining bees and brood or a queenless package of bees added to the damaged colony to strengthen the population. If above mentioned points are kept in mind, the beekeepers can protect their bees to a great extent from toxic effects of insecticides.

Table 14.11 Distance water droplets drift while falling 10 ft in winds of 3 miles/h

Droplet diameter (microns)	Particle classification	Drift distance (ft)
30	Cloud	500
100	Mist	50
200	Drizzle	16
500	Light rain	7

14.26 Managing Pesticide Drift

Serious honeybee poisonings can be caused by pesticide drift. During warm periods, large numbers of honeybees may mass on the outside of the hive to help control hive temperatures. These exposed bees can be destroyed easily by drifting pesticides. Drift control is vital during every pesticide application. Several techniques can be used to reduce the possibility of drift:

- Use pesticides that have low volatility.
- Use formulations that resist drift and volatility.
- Use low pressures during spraying.
- Use nozzles which reduce formation of small spray particles.
- Use high water volumes during application.
- Apply pesticides close to the crop or soil surface.
- Avoid applying pesticides when the temperature is high.
- Avoid applying pesticides during windy conditions.
- Use drift reducing adjuvants.

Certain formulations of pesticides can help reduce drift. For example, low-volatile acid and amine 2, 4-D formulations have less potential for drift than ester formulations. Dust formulations drift much more readily than most sprays. Granular formulations are relatively less likely to drift.

Droplet size of pesticides during application is extremely important in determining the potential for drift. The ability of particles to drift increases greatly as the particle size decreases (Table 14.11).

The various spray nozzles and application equipment produce a wide range of droplet sizes. There are several techniques that will reduce the number of the smallest particles while still giving effective coverage.

1. Application pressures are important in determining the sizes of droplets that are formed. As pressure increases, the number of fine particles also increases. Drift can be reduced by reducing sprayer pressures during application.
2. Nozzle construction can also affect the number of small particles that are formed during spraying. Nozzle tips that produce larger droplet sizes help reduce drift. For example, larger nozzles can be used at lower pressures to get the same volume (Gallons per acre, or GPA) as smaller nozzles operated at higher pressures. Use of higher GPA applications are an alternate means of achieving adequate crop coverage with minimal pressures.

3. “Thickening” or “drift control” adjuvants can be added to the spray mixture to reduce drift. These compounds can increase the percentage of larger droplets which are formed but do not completely eliminate small droplets.
4. The weather conditions during application have a great effect on pesticide drift. Air movements, both horizontal and vertical, cause pesticides to move away from where you are spraying. The higher the wind speed, the larger the amount of pesticide that will be carried away.
5. Pesticides should never be applied during high wind conditions (greater than 10 mph). This is particularly important when wind direction is likely to move drifted pesticides onto nearby sensitive crops or other sensitive areas. Drift to sensitive areas often can be avoided by spraying when the air is moving away from these areas.
6. Drift may also increase when warming air near the soil rises. Applications should be done at times when air and soil temperatures are most similar, often during early morning and late evening. At this time, vertical air movements are lowest.
7. If the air near the soil surface is cooler than the air above, an “inversion” exists. Small spray particles remain suspended in the cool air during temperature inversions, and the particles do not settle readily onto soil or plants. Later the suspended particles move out of the crop on winds and drift. Pesticide applications should be avoided during inversions.
8. Temperature and humidity can affect pesticide drift. When the temperature is high and humidity low, particles evaporate most rapidly. This evaporation causes droplet sizes to decrease and drift more readily. Volatile pesticides also evaporate more rapidly with high temperatures. Pesticides should be applied when the temperature is cool.
9. Height and orientation of sprayer nozzles can also affect drift. Distance and time for spray droplets to reach plants or soil is directly related to the height at which a pesticide is released. Sprays should be released as near the target as will permit adequate coverage. Sprays should also be directed so droplets are propelled downward to reduce the distance of droplet fall. Vapor drift of soil-applied pesticides can be reduced by properly sealing the soil after application. This often involves proper soil incorporation of the pesticides during application.
10. Avoiding pollution of ground and surface waters.
11. Contamination of ground and surface waters is an imminent hazard of using pesticides in agriculture. This potential must be a major consideration in planning for pest control on cropland and other agricultural areas. Elements that enter into water pollution by pesticides are:
 - (a) Proximity of the treated area to surface waters.
 - (b) Proximity of the treated area to drinking water wells or aquifers.
 - (c) Depth of the water table at the treated site.
 - (d) Soil conditions that increase the potential for the pesticide to leach into ground water.
 - (e) The hazard of the pesticide as a potential contaminant of ground waters.
 - (f) Conditions during application that affect pesticide drift into surface waters.
 - (g) Crop management practices that minimize pesticide leaching.

- (h) Precautions during application to avoid leaching or direct ground water contamination.
12. Irrigate in a manner that reduces pesticide movement. High rates of irrigation can increase the amount of pesticide leaching. Excessive irrigation can also cause run-off and erosion. Particular care should be given when irrigating shortly after a pesticide application, since the pesticide is in the highest concentration at this time.

14.27 Honeybee Indemnity Program

Some governments have enacted national legislation to partially repay each beekeeper for pesticide-killed bees. Beekeepers who exercised reasonable precautions to avoid pesticide damage but still lost bees could apply for indemnity payments after January 1, 1967. The main goal of this program was to aid the bee industry in remaining financially stable and to ensure that enough strong colonies would be available to pollinate agricultural crops nationwide. To accurately assess the loss, beekeepers should maintain detailed records of their colonies, noting by date items such as colony condition, population size, syrup and pollen feeding, and honey production. The more detailed the records, the easier it is to establish the true magnitude of a loss and receive reasonable compensation. Be prepared to manage the hives for queen failure or superseding problems which may occur a number of weeks after the pesticide problem occurred.

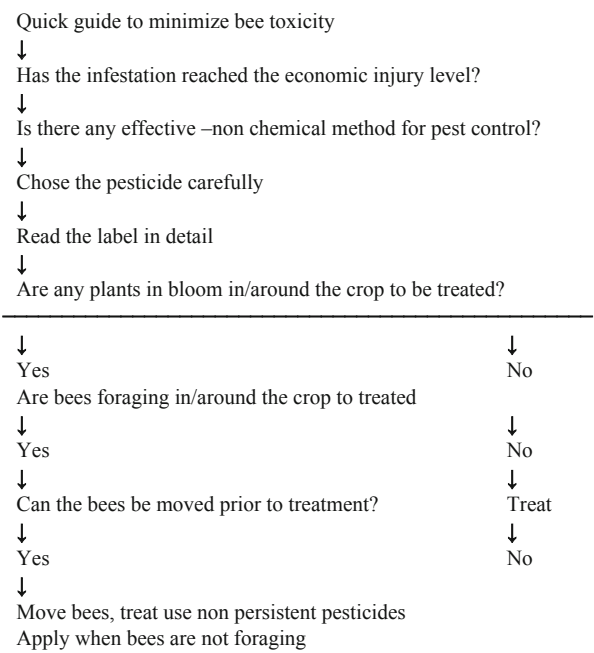
14.28 Plant Poisoning

Poisonous plants can be a problem under certain conditions in limited areas. If a plant's nectar is poisonous, the symptoms of plant poisoning are limited to the blooming period of the plant. However, if the poison is in the pollen, the symptoms may linger as long as the pollen remains in the combs. There is no clear-cut method for differentiating between plant poisoning and pesticide poisoning. The effects of plant poisoning are usually more gradual and last longer than the effects of pesticide poisoning. Plant poisoning usually occurs in the same geographical area at the same time each year, whereas pesticide poisoning is indiscriminate. For a good review of poisonous plants, see Barker (1978). Some examples of plant poisoning are listed below and in Table 14.10.

14.28.1 Purple Brood

Purple brood occurs when adult bees collect and use the pollen and nectar from *Cyrilla racemiflora* (titi, southern leatherwood). This "disease" is characterized by the blue or purple color of the affected larvae.

Fig. 14.3 Flow chart diagram depicting various steps to minimize bee toxicity



14.28.2 Paralysis

Aesculus californica (California buckeye) is probably the best known of the poisonous plants in the United States. Field bees exhibit symptoms similar to those of chronic bee paralysis; i.e., the bees are black and shiny from loss of hair and they tremble. Also, either the eggs do not hatch or the larvae die soon after hatching.

14.28.3 Milkweed Pollinia

The pollen of milkweed (*Asclepias* species) is produced in pollinia (coherent pollen grains) that are attached in pairs by a slender filament. When removed from a flower, the pollinia resemble a wishbone with pollen masses hanging from the ends. Honeybees become ensnared in the thin pollinia attachment and free themselves by pulling the pollinia from the flower. Honeybees often become seriously encumbered and unable to effectively fly or crawl because of the structures that remain attached to their body parts.

Adey et al. (1986) has given the following guide to minimize bee toxicity (Fig.14.3)

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Chapter 15

Management Problems

15.1 Introduction

A honeybee colony will provide the beekeeper with substantial amounts of honey on only one condition: that it is placed in a good foraging area. All other colony manipulations can serve only as complementary approaches, designed to enhance the colonies' efficiency. Past unsatisfactory results in Asian beekeeping development have been primarily due to misconceptions regarding colony management. Whereas the simultaneous blossoming of both cultivated and wild flora during the spring and summer offer great opportunities for temperate-zone colonies to collect and store their food, this situation does not always apply in the tropics. The natural vegetation pattern in tropical Asia is generally irregular, with diversified populations of many floral species. The simultaneous blooming of a single good forage source, or of only a few, covering large land areas, is a rare phenomenon in undisturbed tropical zones; it is mostly found in cultivated or otherwise disturbed lands, where one or a few species become dominant. The success or failure of colony management with *Apis cerana* depends largely on the beekeeper's ability to find good bee forage land and to adjust his colony manipulation techniques accordingly. Management of honeybee colonies requires their timely inspection and then meeting the requirements, so that they can work effectively for honey production as well as pollination.

15.2 The Inspection of Honeybee Colony

One of the greatest advantages of modern hives is that a colony can be examined thoroughly to know the working of it and determine problems if any. The examination of the colony makes it easy to promptly meet its needs. However, the beginner must open the hive less frequently and only when it is absolutely necessary. Unnecessary interference and sometimes mishandling of the bees during inspection makes the colonies to abscond. The bees do not like too much interference, as it upsets their normal working. Therefore, they should be disturbed as little as possible. During the build up or honey-flow period, the bee colonies need to be examined every week

and during the off-season only once or twice a month a colony may be examined when, the bees are busily engaged in nectar or pollen collection. In summer, they should be either examined in the morning or evening, but not during day time. During winter, they should be examined in the middle of a warm day. A hive should not be opened on a windy or rainy day or at night when the bees are not flying in and out. In winter, the brood frames should not be exposed to cold too long and the hive should not be opened too often. During a visit to the colony, the beekeeper should not be perspiring profusely or strongly smelling of alcohol. Before approaching the bees for inspection, he or she should put an overall, a bee veil and if required gloves also. Then he or she should proceed as described in the following paragraph.

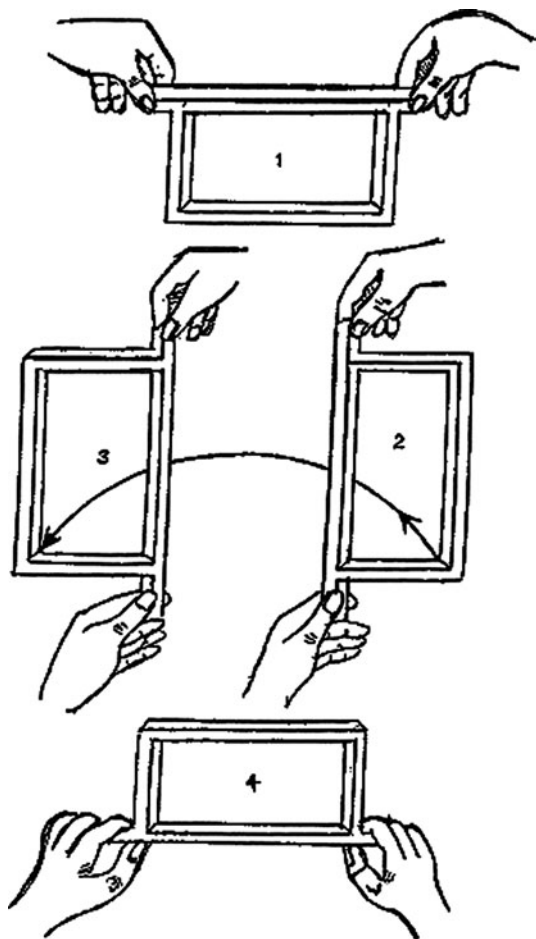
After lighting the smoker (rags, or cotton waste, wood shavings, dried cow dung, maize cobs, pine cones, dry leaves, etc. make good fuel, for smoker) the hive should be approached from side avoid interfering with bee's flights. A few puffs of smokes should be given at the entrance. Lift the inner cover gently and blow a few puffs of smoke below. Take care and avoid quick jerky motions. Then the frames should be pried apart with the hive tool and taken out one by one. They should stand in a vertical position and preferably over the hive. For examining, the other sides of the combs, it should be manipulated as shown in Fig. 15.1. After examining, replace them in the brood chamber in the same order they were found. During these manipulations, queen should always be kept in mind and avoid crushing her. The frames should be handled gently and jerking and crushing of the bees must be avoided, so that they are not provoked to sting. As apart from being painful, the odour of the poison irritates the bees even more and makes them difficult to handle. If a bee stings you, the visible sting should be pulled out with the sharp edge of the hive tool or knife and not with the tips of your finger. After finishing the work, the hive should be closed at once. The cover should be put on in such a way as not to crush any bee. Keep the record of every colony and enter your observations in the data ledger. This record will help you to tackle the needs of the colony.

The aim of the colony inspection is to determine whether the requirements of the colony are being fulfilled at that particular time which would lead to an increase in production.

When a colony is examined the following points should be taken care of:

1. Whether the bees have sufficient stores of nectar, pollen, or require artificial feedings.
2. Whether the eggs are present and properly laid in a regular pattern. This will indicate that the queen is well and there is no need to search for her. But, if there is no egg, then search must be made to locate her. Some beekeepers have marked queens on thorax to make the visibility easy.
3. Examine for the presence of queen cells, destroy if any, and crush the larvae or eggs. But, if the colony is preparing for swarming, allow it to rear queens.
4. The presence of queen cells during off-season indicate that either the queen is missing or old queen is not working properly and workers intend to supersede it. (Supersedure means replacement of old queen with new prolific queen).

Fig. 15.1 Manipulating a frame: 1 normal position, 2 held at right angle, 3 after revolving 180° and 4 the outer side of the frame



5. Determine whether enough empty combs are available for queen to lay eggs and for the bees to store honey. If sufficient combs are not available, more frames should be provided.
6. Examine if any enemies or diseases of the bees are prevalent. If you notice any symptom of disease, take precaution and remedial measures immediately.

15.3 Transferring Bees from Feral Nests

Despite the fact that transferring *A. cerana* colonies from feral nests to movable-frame hives is time consuming, its cost is low and it is practical for villagers with spare time. The beekeeper can learn of the presence of a feral nest in one of several ways: by active search, often in areas already known to have been frequented by bees; by tracking

foraging bees back to their nest; or by word of mouth. Some beekeepers pay a small reward to informants, mostly village children, who report the presence of accessible feral colonies. Once a colony has been located, the operation is quite simple. The equipment required includes a knife, a hive tool or other means of opening the nest, a hive with empty wired frames, a smoker, a roll of twine, a brush and a litre of 1:1 sugar syrup in a vaporizer. If available, a portable battery-operated comb-embedder is useful. The beginner beekeeper will need good protective clothing and a veil, but as he gains experience and learns to avoid disturbing the bees unnecessarily, he can dispense with some of this protection. The best time of a day to transfer bees is late in the afternoon, when flight activity is minimum. The beekeeper first applies a little smoke at the hive entrance, and then gently opens the nest wall, exposing the combs. After spraying sugar syrup on the bees until most of the workers are coated with it, he cuts all the brood and honey combs from the nest. He embeds the combs in empty wired frames by gently exercising pressure on them until their mid-ribs reach the wire; this operation is facilitated by heating the wire, if the necessary equipment is available. He further secures the combs to the frames by tying them with twine and then places the frames in the hive, brushing as many bees as he can into the hive with them. It is most important that the queen be with the colony in the new hive. It is useful to find her and place her in a queen cage, to ensure that the workers remain in the hive. When all is done, the transferred colony is moved several kilometres from its original site, to prevent some of the workers from drifting away. If possible, two or three frames of honey should be given to the newly transferred colony. The queen should remain caged for 3 or 4 days (or a week at most) before being released to resume its egg laying.

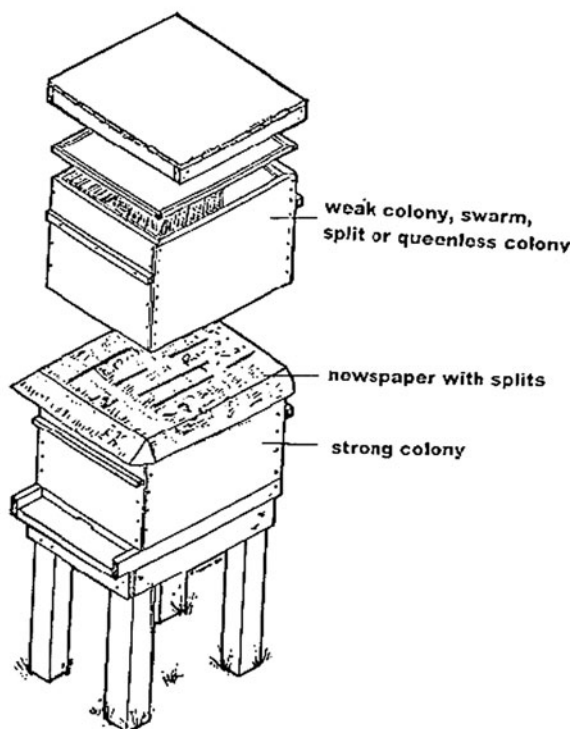
15.4 Uniting Honeybee Colonies

In beekeeping operations, sometimes there is need to unite the weak colonies which are unable to sustain independently. The chances of survival of weak colonies increase if such colonies are united and given one queen. In some circumstances, when a colony loses its queen and has the tendency to become the laying workers. To overcome such a situation, it becomes essential either to provide new queen or immediately unite with other colonies. Whatever may be the reason, but uniting procedure remains the same as illustrated in Fig. 15.2.

Each colony has its own colony-specific odours and it is very difficult to combine the two colonies unless their odour is mixed well. Any attempt to unite these colonies without mixing their odour result in infighting and deaths will occur on large scale. Therefore, the first step will involve bringing the two colonies into contact with each other. If uniting is done abruptly, the field workers of the shifted colony will not recognize the new place and returning to their original place will persist. This problem can be overcome by moving a hive gradually at the rate of 2 or 3 ft per day, so that the field bees get accustomed to the changing position of their hive and will not drift back to the old site. When colonies are sufficiently close, one or

Fig. 15.2 Uniting honeybee colonies by news paper method

Uniting colonies-Newspaper method



two feet apart, they are ready for uniting. They can be united by following either newspaper method. The top cover is removed and the frames are covered with a piece of newspaper having a few holes made with a small nail the bottom, board of the upper colony is then removed and the brood chamber, is placed above the other colony, the newspaper forming a partition between the two. After a day or two, the odours of the colonies will mix and the bees will cut through the paper and will unite together, forming a single colony. After a few days all the frames can be placed in one hive and the upper chamber can be removed (Fig. 15.2).

Alternatively, colonies can be united using smoke method. When the colonies to unite have been brought close to each other, both should be smoked heavily and thin sugary syrup scented with oil of peppermint or wheat flour sprinkled over them. The combs with the bees of the colony to be united should be altered with the combs of the other colony. More smoke and syrup or flour should be applied and the colony should be closed. The work of the queen may be checked up after 3 or 4 days.

It is better to unite a laying worker colony to several strong colonies by giving from one to two frames to each of them. If all its frames are united to one colony, then there is danger of latter's queen being killed by the laying workers.

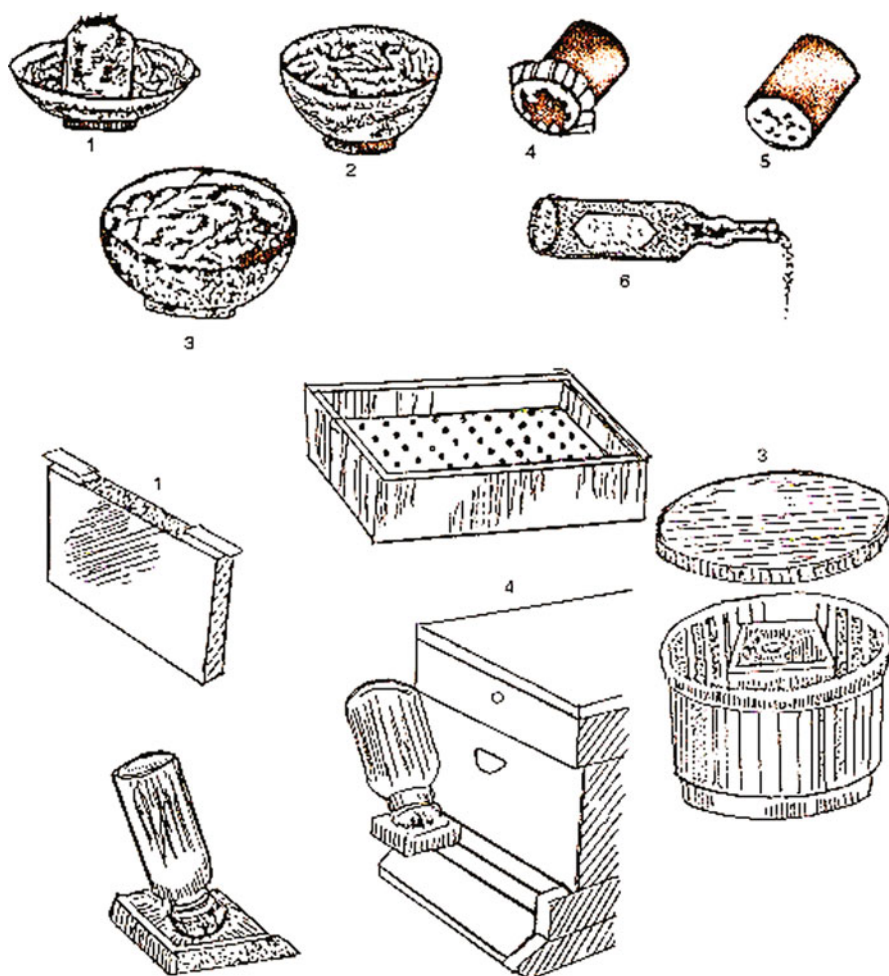


Fig. 15.3 Feeding apparatus. (*Upper*) Indigenous feeders: 1, 2 and 3 cup feeder with cloth and glass inside; 4, 5 tin feeders having cloth wrapped on the mouth and small holes in the bottom and 6 bottle feeder. (*Lower*) 1 Division board feeder, 2 flat feeder, 3 rapid feeder and 4 boardman feeder separately

15.5 Artificial Feeding of Honeybees

Honey is the best food for the bees and a beekeeper should always leave enough stores for them at all times. However, during dearth periods artificial feeding becomes necessary (Fig. 15.3). When honey is not available, the best substitute is sugar syrup made from white crystalline sugar by dissolving 2 parts of sugar and 1 part of water or 1 part of sugar and 1 part of water. The use of jaggery, molasses, etc. is not recommended as these substances ferment in the cells and also cause dysentery. The

sugar syrup can be fed to the bees in small dishes with floating straws. The bees will not be dipped in sugar and feed while sitting on these straws. The feeding should not be done during the day time which may result in robbing. It should be done in the evening hours. During early spring, the feeding of the bees is done to stimulate them that season has started. The queen starts laying more and more eggs before the actual flora is available. This type of feeding is called as stimulative feeding to prepare the colony to utilize the nectar-flow season.

Feeding of sugar is must to survive the colonies during dearth periods, but if the feeding lasts a long time, the functional activity of bee is negatively affected, they winter more poorly and their productivity is greatly reduced. Experiments have shown that honey feeding is the best, honey mixed with sugar is of secondary importance and sugar alone as the lowest quality food. It is therefore recommended that while extracting the honey, sufficient honey stores be left for bees. It has been found that disturbance in animals nutrition diminishes their enzymatic activity, weakens the defence of organism and brings about various pathological changes.

15.6 Pollen Feeding

Pollen is a protein food. Brood rearing is impossible without it. Therefore, there is a natural direct dependence between the arrival of pollen to the nest and the amount of its brood. Pollen such as honey must be available in adequate quantities to a wintering colony, otherwise spring dwindling occurs due to insufficient pollen stores. A wintering colony must replace its fall population with young bees and have a large active brood nest by the time natural pollen is available in late winter or early spring. Whenever there is a pollen shortage it can be provided in two ways: pollen substitutes and pollen suppliments.

15.6.1 Pollen Substitutes

Pollen substitutes are prepared using the following ingredients:

Ingredients	Quantity in gram
Wheat flour	100
Soya bean flour	100
Skimmed milk powder	20
Dried brewer's, yeast	25
Sugarcane	60
Fat or edible oil	50
Sodium biocarbonate and	50
Cream of tartar (1:1)	5

All the ingredients are mixed together and kneaded to a soft dough before being served to the bees.

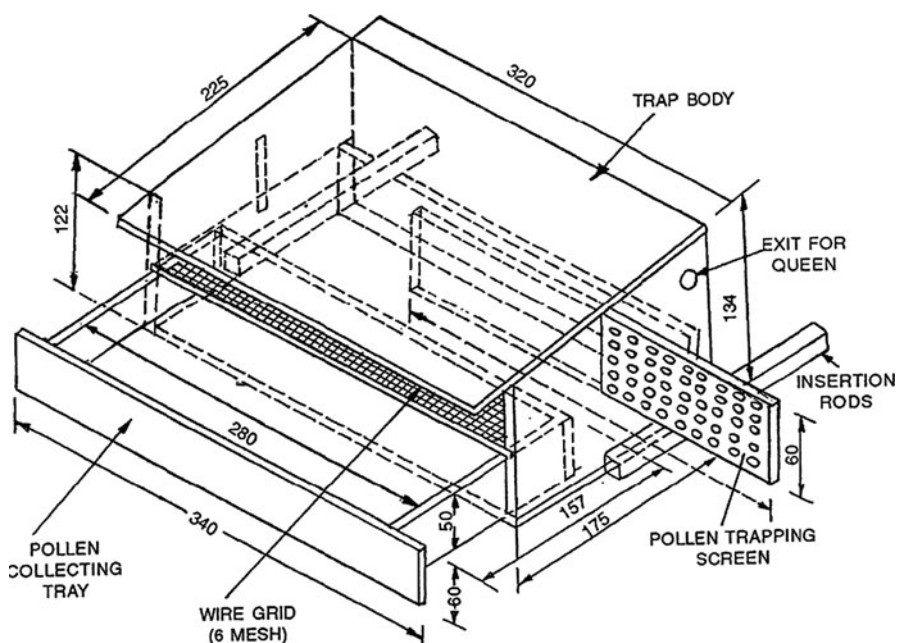


Fig. 15.4 Isometric view of a pollen trap (all dimensions in mm). (Redrawn from Chhuneja and Goyal 1996)

15.6.2 Pollen Supplements

Pollen is collected manually from flowers or by bees using pollen traps when available in abundance and is stored for future feeding. A pollen trap is used in front of the hive entrance. The bees while passing through the grid, lose the pollen they carry, the pollen pellets drop onto the grid and down into the pollen trap (Fig. 15.4) without hindering in any way a colony's growth its preparation for winter, as well as its honey harvesting, one can obtain from it as much as 6–8 kg pollen within one season.

A pollen trap can be locally fabricated (Chhuneja and Goyal 1996). It is made of kail Wood (*Pinus wallichiana*) and its construction details are illustrated in Fig. 15.4. It consists of a plastic strip of 1.5 mm thickness which serves as a vertical pollen-trapping screen in which circular holes of 4, 5 mm diameter are made. The pollen balls in the corbiculae of foragers get detached when such bees pass through these holes. The plastic strip contains 5 rows of such holes (28 holes/row) with a working area of 28.2×6.0 cm. The balls detached from the corbiculae are collected into wooden pollen-collecting tray kept below a 6-mesh wire grid on which the pollen foragers alight before entering the hive. The trap has two compartments. Lower compartment is comprised of a drawer-type pollen-collecting tray. It is enclosed from its back and sides by the trap body and from the front by a wooden strip attached to the tray for facilitating its easy removal. The tray ($28.0 \times 15.7 \times 5.0$ cm) is covered at its top by

the wire grid (6-mesh) which serves as an alighting board for the bees, preventing the bees from taking away the collected pollen from the tray and also preventing the dead bees falling into the tray. The upper empty compartment is enclosed from back by the plastic-trapping screen and from top and sides by the trap body. The trap body is in the form of a trapezium, the top plank with a little downward inclination towards the front. Its internal length is 28.2 cm, whereas internal width is 17.5 cm. Its internal height at the rear end is 11.4 cm, whereas it is 10.2 cm at its anterior end (upto the trap body). Beyond the trap body is a top plank extended by 5 cm in the form of an eaves, so that the rain showers should not enter the trap and spoil the pollen. Leaving a space of 2–7 cm from the rear end and 8.7 cm from the bottom, one hole of 7 mm is made on each of its side plank. Such holes facilitate the exit of drones and the queen bee for nuptial flight. Leaving a space of 9.5 cm from the front, two wooden rods ($17.5 \times 2.22 \times 2.22$ cm) are fixed one each at the bottom of each side plank, so that these rods extend by 9.6 cm from the rear end.

For installing the trap on to the hive, the front wooden entrance rod is removed from the hive and the trap is fixed to the hive by inserting the extended portion of the rectangular insertion rods of the trap into the hive through the space so created after removing the entrance rod. Pollen-trapping screen should be kept a little displaced from the exact position for the first 2 days, so that bees could pass through without dislodging their pollen loads and get accustomed to this new attachment; otherwise bees show a considerable reluctance to pass through the pollen-trapping screen and remain disturbed for a long period of time. After the bees become used to pass through the trap, the screen is fixed to its exact position. The bees then readily accept it and start passing through the pollen-dislodging holes. The pollen so collected can be fed to the bees during pollen-scarcity periods. Yields per hive vary according to the plant source, colony strength, weather and type of the trap.

The pollen is also collected manually *from* maize and other sources when abundant. During pollen shortage it is *fed* to the bees along with following ingredients:

Soya bean flour	3 parts
Pollen	1 parts
Sugar syrup	2 parts
White of an egg	1/20th parts

All these ingredients are mixed thoroughly and fed to the bees.

15.6.3 Pollen Storage

The collected pollen can be stored for long periods. Pollen may be stored for 3 days in refrigerator having + 4 C temperature for long storage, its moisture content must be less than 20 %. The lower the moisture content, the greater will be its storage quality, and pollen may be dried in automatic thermoregulators maintained at 40–42 C. When the pollen is ready, that is, its moisture content is reduced to 10–12 %, it

is immediately packed in polythene bags, milk cans or glassware. It is very important to have the pollen tightly sealed, so that it does not contact air and absorb moisture during storage.

Pollen can retain its qualities if it is mixed with honey/sugar syrup and stored. When preserved with honey, the proportion is 2 parts honey to 1 part pollen; when preserved with sugar powder, the proportion of dried pollen and sugar powder is 1:1. Pollen so preserved is stored in some dark and cool place.

15.7 Moving Bees

Flying bees return to their original location. Bee colonies can be moved by about a meter each day and by steps can be shifted to few meters in the apiary. If a colony is to be moved few hundred yards within few days, the best course is to move the colonies 4–5 km away, keep them there for 5–6 days and then bring it back to the desired place. During this period, the bees will lose the memory of the earlier site in the apiary. Bees should always be moved in the evening or night.

15.7.1 Transporting Bees

Transportation of hives to a new locality requires great care and precaution. The following procedure should be followed: Move all the frames to one side of the brood chamber and press them tight. Fix one nail on each end against the last frame, so that they remain in position, screen the top of the hive with a piece of wire gauze and nail down all the movable parts making the hive completely bee proof. After dark, when all the bees have returned home, close the hive entrance with a piece of wire gauze; the hive is now ready to be transported to any place. In hot weather or during a long journey, water should be sprinkled daily on the screen. There should be enough food for the bees to last during transportation or couple of days after transportation. Travelling in hot weather should preferably be done at night. On arrival, the hives should be placed at their new sites and their entrance screen should be removed. After sometimes when the bees have oriented themselves, the top screens may also be removed and the colonies examined to remove any damaged or broken comb. A further examination may be conducted after a day or two to assess the working of the queens.

15.8 Comb Space

It is very important that bees should always have sufficient comb space to work, for the queen to lay eggs and expand the brood area. The additional space is provided by giving the raised frames and *if* they are not available by offering frames fixed with comb-foundation sheets. When the brood chamber is full, a super or honey chamber should be provided.

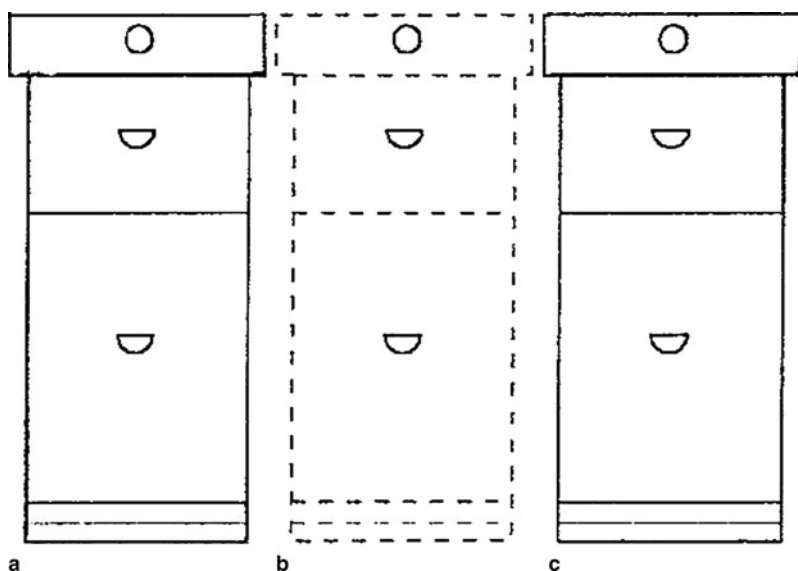


Fig. 15.5 Dividing at the same place: **a** original colony; **b** and **c** divided colonies

15.9 Increasing the Number of Colonies

Experience shows that the *A. cerana* has a high swarming tendency and the number of colonies can be increased by hiving the swarms. Capturing the swarms and hiving them is tedious and risky job as some of the swarms may move far away from apiary site and get lost. It is therefore always advisable that the strong colonies may be divided into two or three equal parts in the spring season when plenty of drones are available.

A colony can be divided into two equal parts: Half of the frames (comb; containing a minimum of 1 frame uncapped brood with one frame honey and pollen each) adhering bees and the old queen is established in a separate hive and moved to a distance of 4–5 km and kept there for a week or so and then brought back to the apiary. The queenless part is kept in the apiary at the site and is given a ripe queen cell or a mated/virgin queen (Fig. 15.5).

In another method, one-third of the frames covered with adhering bees along with old queen are removed and placed in a separate hive. This queen right portion is kept at the old site and the parent colony is shifted to a few yards away in the apiary and established there. The colony at the old site is augmented by some of the returning field bees from the parent colony. The queenless portion (parent colony) is given a ripe queen cell a virgin or a mated queen.

The European honeybees, *Apis mellifera* swarms less and thus maintain strong colonies. In their case, division of the colony should be made immediately after the honey flow. The colonies can also be divided before the honey flow, without

impairing their productivity. Two or three emerging brood combs, with adhering bees but without a queen are removed from a strong colony and placed in another hive. This new colony is shifted to a new site in a apiary and given a ripe queen cell. The older bees in the new colony will return to the parent colony, leaving behind the young and emerging bees. This method does not impair the productivity of the parent colony. The queen in the new colony will mate in due course and the colony may be strengthened by giving bees or brood from the parent colony after the honey flow. When the colonies are rearing brood at a faster rate than normal and there are chances of the “swarming instinct” becoming dominant in the honey-flow period, one or two frames with capped brood along with the adhering young bees (as there is no fighting among young bees from different colonies) are taken each from several colonies and placed in a new hive in the apiary. A queen cell in a protector or a queen or a queen in an introducing cage is put in the new hive and work of the queen checked up after 10–16 days. This method helps to check swarming, but does not weaken the colonies. The bees are able to take full advantage of honey flow.

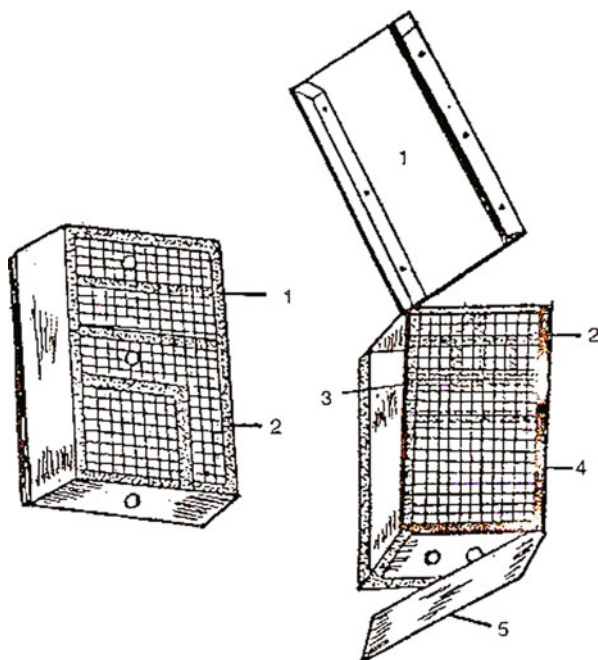
15.10 Method of Queen Introduction into a Colony

The introduction of a queen is required either when there is no queen in the colony or when the old queen is replaced by a new one (supersedure). So, the queen is the heart of the colony, it is the most important organ. The stronger and more fertile she is, faster grows the colony. A queen with good inherited properties, upon mating with hybrid drones, will produce offspring of high productivity; the power of any colony is determined by the youth of its queen. If the old queen is to be replaced by a new queen, the colony is first made queenless, then new queen is installed. If a colony has been queenless for some time and the bees have become laying workers, then the new queen will not be accepted. Under such circumstances, the colony should be united with a normal one.

The first step towards introduction of queen is to destroy all the existing queen cells in the colony. A queen is then placed in a queen cage along with few attendants or worker bees. The entrance hole of the cage is plugged with candy prepared by kneading some sugar powder with a little honey. The cage is then hung in between two frames (one of the frames preferably be having brood) of the queenless colony to enable the queen to acquire the hive odour. The bees will catch up the candy and the meantime, has acquired the odour of the hive. Her work may be checked up after about a week.

If the queen is not accepted and the bees are hostile towards her, she should be re-caged and suspended in the colony as before and should be again released after 24 h or so. Some colonies especially, *A. mellifera* are very sensitive and resist re-queening and thus take longer to accept a new queen. So, the queen be kept in the cage till the aggressive attitude of the bees towards her ceases and she is accepted. If the bees are building the queen cells in the presence of the caged queen in the colony, they should be broken. If possible, all the brood combs from the colony may

Fig. 15.6 a Queen cages.
Left—introduction cage: 1
 food chamber, 2 bee chamber.
b *Right*—mailing cage: 1
 cover, 2 food chamber, 3
 water chamber, 4 bee
 chamber and 5 cover



be removed and placed in another colony. This manipulation helps to bring about a speedy acceptance. If the queen is not introduced by using the above method, then a “push-incage” which consists of a wooden frame 20×13 cm, made of 1 cm thick wood on which a piece of wire cloth with apertures of 3 mm^2 is used (Fig. 15.6). This cage is fixed on the emerging brood comb after shaking of the bees. The queen is placed on the comb and the cage is pushed in position over her, so that only newly emerging bees are inside with the queen, the cage is held in position by means of rubber bands. The cage provides plenty of room for the movement of the queen and she cannot be attacked by older bees of the colony. When the queen starts laying eggs inside the cage, she may be released and at that stage she will be accepted. This method works under all circumstances.

15.11 The Queen-Mating Problems

The queen-mating problems occur during certain parts of the year when drones are not produced by other colonies or they are scarce. Under these circumstances, virgin queen fails to mate and lay unfertilized eggs. Sometimes, the queens are also picked up and devoured by the birds while they are on nuptial flights and, thus, they fail to return. To overcome these problems, it is suggested that colonies should be divided or re-queened only in the spring season when sufficient number of drones are available. In the beginning of spring season, the colonies should be given drawn combs with the

maximum number of drone cells. Some surplus queens who get mated in spring may be kept in small colonies maintaining two or three frames for emergency re-queening. The queen-mating yards should be established where there are no problems of bee-eating birds. It may further be noted that drone of *A. cerana* cannot mate with the queen of *A. mellifera* and vice versa. The colonies of honeybees must be carefully examined for the presence of varroa mites. It has been found that varroa mites attack drone brood, creating drone depression resulting in mating problems.

15.12 Robbing in Honeybees and Its Control

Bees have strong tendency to search and collect sweet substances to their hives. This tendency often leads them to steal honey from weak colonies and especially when there is a dearth of bee flora. The robbers enter through holes, cracks and crevices other than the main hive entrance. Robbing once started is very difficult to control. In severe cases, the queen of the robbed colony is killed and many bees die in their life and death battles with the robbers. The colonies which are not strong enough to repel the invaders are robbed, ruined and ultimately abscond. Owing to the loss of hair in fighting, the robber bees become smooth, shiny and almost black. The Indian honeybee *A. cerana* is very prone to robbing. The European bee *A. mellifera* also robs *A. cerana* and vice versa during dearth periods and it is very difficult to keep both the types of colonies together in an apiary. As soon as the robbing is detected, the entrance of colonies should be narrowed down to one bee space. Wet grass or a piece of cloth soaked in diluted carbolic acid or kerosene, may be put near the entrance of the hive of the robbed colony. Efforts should be made to locate the colony from which the robber bees are coming. To identify, the entrances of all the colonies (except the colony being robbed) should be closed. Fine wheat flour or talcum powder may be sprinkled over the alighting board of the robbed colony. Thus, marked bees could be easily located at the entrance of the colony, from which they come. After identifying the robber colony, the entrance of other colonies should be reopened. The robber colony or the robbed colony may be shifted some miles away.

It is much easier to prevent the bees from robbing than to fight it, when robbing takes the shape of an assault; it is very difficult to quench, and sometimes even impossible.

Bees, invading the nests of others; may spread any infectious disease which they may be carrying on their bodies. But, it is more common that invaders themselves get infected by drinking infected, honey or coming in contact with stranger's nest and foreign bees thus bringing infections to their home.

Bee robbery is an extremely hazardous evil. To prevent it following precautions should be taken:

- Do not examine colonies during dearth periods, if necessary proceed very quickly and do not keep the honey or other combs exposed for long.
- Do not expose honey or any other sweet material in the apiary.

- Feeding with sugar syrup is done in the late evenings, always inside the hive and never outdoors.
- Honey should be extracted in a bee-proof room.
- Minimize all chances of robbers gaining entry into the hive. Entrances can be reduced, so that guard bees can defend effectively.
- Do not drop even a single drop of honey when withdrawing it from the hives.
- Try to keep the colonies strong with large stores of honey as the robber bees more frequently attack the weak colonies.
- Do not open the nest completely, especially at the time when there is no honey harvesting.
- Badly robbed colony should be moved to a new place and an empty hive placed at its place.

15.13 Seasonal Management of Honeybee Colonies

The management of honeybee colonies depends upon the climatic and floristic conditions in a locality. In view of the varied climatic and floral conditions of the country, there cannot be one standard management that could be adopted for multiplication of honey production. Management practices round the year in subtropical, intermediate and temperate areas are given in the Table [15.1](#).

15.13.1 Winter Management

Proper management practices for successful overwintering of the colonies depend upon the following three important factors:

1. Adequate population of young bees.
2. Sufficient stores of food.
3. Protection of colonies, especially from moisture and cold.

15.13.1.1 Adequate Population of Young Bees

Prior to the commencement of winter, each colony should have a large number of young bees. Young bees are not only capable of surviving the winter, but are also essential in starting good brood rearing soon after the winter, that is, in early spring. Dwindling of colonies during and after winter is mainly due to the presence of proportionately greater number of old tired bees who had work heavily during autumn flow. These bees die soon; even those which may survive winter, collapse more rapidly in early spring. The rate at which new bees are produced in spring is slow compared with the heavy death of old bees. The condition of the colony becomes precarious if there are other factors such as inadequate stores, lack of proper

Table 15.1 Beekeeping management practices round the year

Name of the operation	Outward symptoms	Sign and subtropical areas	Intermediate	Temperate areas
Removal of outer winter packing	Warming up of weather and the blooming of earliest spring flowers such as peach, plum, apricot sarson and wild pear	Generally no outer packing is given	If packing is given, remove it after the middle of February; clean the hives	Outer packing should be removed in the end of February or early March and the hives should be cleaned
Removal of inner winter packing	The lengthening of days; further rise in day temperature; blooming of spring flowers	The inner packing should be removed after the middle of the February or according to the prevailing weather conditions	The removing of inner packing in the February or first week of March according to weather conditions	Inner packing should be removed after the middle of the March according to the prevailing weather conditions
Simulative feeding	The opening of spring flowers; increased bee and brood activity; abundance of nectar and pollen plants	If need, simulative feeding with 35 % sugar syrup in the beginning of February to stimulate early brood rearing be given	Feed the bees in the middle of February with 35 % sugar syrup to stimulate brood rearing and again the middle of March to ensure rearing	Feed the bees in early March with 50 % sugar syrup according to their needs. Give terramycin in sugar syrup
Control of the acarine disease	Observe the symptoms, if K-shaped wings and crawlers are available, then acarine disease is present	Segregate the diseased colonies and give them "Folbex" treatments. Other colonies be treated in Feb. or March as a preventive measure against disease	Segregate the diseased colonies and give "Folbex" treatments. Healthy colonies should be fumigated with "Folbex" in April as a preventive measure against the disease	Segregate the diseased colonies and treat, them, with "Folbex" treat healthy colonies with "Folbex" in the middle of March or April as a preventive measure against the disease
Strengthening of weak bee colonies	Weak colonies covering less than three combs	Strengthen the weak colonies by giving them sealed brood frames in February–March	Unite exceptionally weak colonies with strong ones in the middle of February; strengthen weak colonies by giving sealed brood in March	Weak bee colonies may be given sealed brood frames from strong colonies in March–April. Exceptionally weak colonies may be united with strong ones in early March

Table 15.1 (continued)

Name of the operation	Outward symptoms	Sign and subtropical areas	Intermediate	Temperate areas
Providing comb space	Increased pasturage for bees; extensive brood rearing and overcrowding	Give new empty frames for egg laying and give frames having comb foundation sheets on outer sides	Make sure that queens are never short of the egg-laying space; provide comb space whenever required	Provide sufficient space for queen to lay eggs by adding more drawn combs or frames fitted with comb foundation sheets during the building up period in spring to relieve congestion in the hive
Swarming and swarm control	Overcrowding the brood nest raising the drone brood and building of the queen cells. Bees may swarm soon after the first queen cell is sealed over	Keeping hives ready for hiving the swarms. Control swarming in April. It is good time to start beekeeping for beginners	Keep beehives ready for hiving the swarms after the middle of February. Control swarming in April. This is a good time to start beekeeping for beginners	Keep bee hives ready to hive swarms in April. Control swarming after middle of May. This is a good season to start beekeeping for beginners
Honey flow and honey extraction	The whitening of honey cells; the appearance of brace and burr combs; an increase in the weight of colonies	Bees gather surplus honey from shisham citrus Eucalyptus, litchi, Sarson and berseem flowers. Honey is generally extracted at the end of April of in May, depending upon the locality and opening of the flowers	Provide proper space for honey storage. If brood chamber is full, provide super. Surplus honey is stored from Tun, Puna, shisham, Eucalyptus, soapnut, etc. Wxtract honey in May depending upon locality	Surplus honey is stored from barberry, false acacia, clovers, wild rose horse chestnut, etc. Control swarming during the honey flow. Provide space for honey storage. Extract surplus honey in June. Leave enough honey for monsoon brood rearing

protection from moisture, cold, infestation with diseases such as acarine, nosema, etc. An inferior queen will leave colony very weak and is incapable of surviving through winter. To have plenty of young bees before and after winter it should be seen that the entire colonies are headed by young prolific queen capable of laying satisfactorily till the later part of September. Only such colonies have sufficient young bees for wintering. Every year or every alternate year a new queen should be introduced in a colony in spring or summer, so that they can begin laying and produce enough number of young bees for a good winter cluster. Winter survival of weak colonies is very uncertain. It is therefore desirable to unite such weak colonies keeping the young queen in the colony.

15.13.1.2 Stores of Food in a Colony

Sufficient quantity of honey stores should be left over in a colony for consumption during winter. One super of honey completely filled and sealed is adequate. Honey stores in brood frames and one super on an average per bee colony through winter. Where honey flow is very short, and when there is a little surplus honey gathered by the bees, one may have to feed sugar syrup to the bees for winter survival to substitute the extracted honey. In such cases, 500 ml by weight may be fed to bee colonies. Feeding should be given in the evening to avoid robbing. Depending upon the strength of the colonies 3–5 kg of sugar syrup (75 % sugar by weight) may be fed to them.

15.13.1.3 Protection from the Humidity and Cold

Honeybees live in a thermal environment of their own and air condition their nests at temperatures between 32.3 and 35.0 °C. This is done at the cost of honey used for fanning in summer and for muscular movements to produce heat in winter. Insulation of their abode helps to reduce consumption of honey and save energy of the bees. At a temperature of 14–15 °C, bees start forming clusters; first indication of formation of winter cluster and approach of winter is killing of drones. This is the time for assuring adequate stores in the colonies and their packing for protection from the cold. It has been established that if requirement under (1) and (2) above are fulfilled, then even light packing is sufficient to ensure the winter survival of bees. Packing requirements further vary with the type of climate in an area. In plains, very light packing is sufficient, but in hilly areas with severe cold a good packing is needed to protect the colonies from cold and keep away the moisture.

15.13.1.4 Reduced Entrance and Adequate Ventilation

Winter losses also occur due to heavy packing. Condensation of water inside the hive disturbs the winter cluster resulting in the loss of the colony. An important factor to be borne in mind while packing the colonies is to provide a reduced entrance at the bottom and a small auxiliary entrance at the super chamber level. Prior to the

packing of colonies, a 13 mm hole should be bored to the super on any one of the sides. The normal hive entrance should be reduced to a small slit of 5 cm wide and 1 cm high. The temperature around cluster is almost equal to the temperature outside the hive. Top entrance permits the bees to fly from the top of the cluster and also allows circulation of the air to help dissipate excess moisture and thus prevents its condensation.

An inner cover with a feeder hole fitted with wire screen is placed over the super chamber and the hive top is placed over it.

15.13.1.5 Winter Packing

Colonies require heavy packing in mountainous areas and operation should be completed well before the first frost of the season (Brar et al. 1987). A packing of chopped rice or wheat straw is placed in the surplus space of the brood chamber. This inner packing is done in brood chamber. This inner packing is further supplemented with an outer packing of straw mattings on all the six sides of the hive. These mattings are tied with a piece of string to protect them from flying away during a storm. If the hives are not under a roof and are lying out in the open, exposed to rain, the outer packing is further covered with a polythene sheet, so that the packing does not become wet and soggy. Thermocole sheets can also be used for packing. Colonies thus packed, are not disturbed during the cold months and their condition is watched from the behaviour of bees at the entrance hole. If something, abnormal is suspected, the colony may be unpacked and examined on a warm sunny day.

15.13.2 Spring Management

Spring is the most important season for the bees. During this season, they increase their brood-rearing activity and build colony strength in preparation for the coming honey flow. When the spring comes, first the outer packing is taken out only when the weather warms up. After removing the packing, the colonies are examined on a warm sunny day. The bottom boards are cleared of all the debris. The condition of the queen is checked. If there is no queen in the colony, it should be united with another one. The honey stores are checked and the colonies having shortage of reserves are given sugar syrup. All the colonies should be fed when the spring flowers begin to appear. Artificial feeding is very important during this period to stimulate the bees to produce the brood and to time their activities. The bees which gather surplus honey during this period are generally old bees. For the new bees to reach from egg to adult stage it takes 3 weeks and for the first 3 weeks she attends indoor duties, it is only after 3 weeks that her field work begins. Thus, the colonies must be prepared for the main honey flow at least 6 weeks earlier. This is how the artificial feeding is helpful in timing the brood rearing, early in the spring. In a certain year, if the weather warms up earlier and the honey flow is expected earlier than usual, the bees

can be stimulated likewise. When the brood-rearing activity starts, extra comb space is provided, if necessary. Weak colonies should be given a few sealed brood frames taken out of the strong colonies. Soon, the colonies gain sufficient strength and the bees feel crowded. In certain temperate areas, inclement weather at the height of the brood-rearing season in spring makes the bees stay indoors. The suspension of the field activities, when the food requirements of the colonies are very high, lead to the death of many colonies owing to starvation. Under such circumstances, the beekeepers should feed the colonies, if there is shortage of food.

15.13.2.1 Swarming

This is the natural process of multiplication of colonies when the strength of the bees in the colony increases and bees feel overcrowding, they make preparation of swarming. When brood chamber is full of pollen, honey and there is overcrowding for queen to lay eggs, a fever or stimulus starts in the colony called as swarming fever. In this state, the queen cells are raised and new queens are reared. When the development is completed, these cells are sealed and in the warmest part of a sunny day, the colony issues a swarm, taking the old queens along with it. It is generally the old queen and young bees who accompany the swarm. The swarm settles on nearby tree or a bush and is caught. If the mother colony is weak, the swarm is returned to the same hive; otherwise it is put in a new hive, kept ready before hand, with raised frames or frames fitted with comb-foundation sheets.

15.13.2.2 Catching the Swarm

Catching a swarm looks rather difficult, but with little practice it turns out to be a very simple operation. The method is as follows: Gently place the swarm basket over the swarm and move the bees with hand, pushing them in (Fig. 15.7). When the bees have entered the hollow of the basket, take it to the apiary and keep it in a dark, cool place. Late in the evening, take the swarm basket to the hive already prepared for the purpose. Remove the inner cover of the hive and place in a slanting position, slopping from the entrance hole down towards the ground. Shake the basket over this board. The bees will move up the slope and march into the hive through the entrance. They will soon occupy the frames and settle down. A brood frame from the parent colony and sugar syrup given at night will further help them to settle. A swarm caught in this way, if found small, weighing less than 3 £, should be united with the mother colony or another weak colony and interface with the production of honey. Only a swarm from an average colony is obtained and kept as a separate colony. The weak colonies should not be allowed to issue swarms.

15.13.2.3 Prevention of Swarming

Swarming can be controlled by relieving congestion in the hive. More frames should be given, the old queen should be changed and shade should be provided. Provide

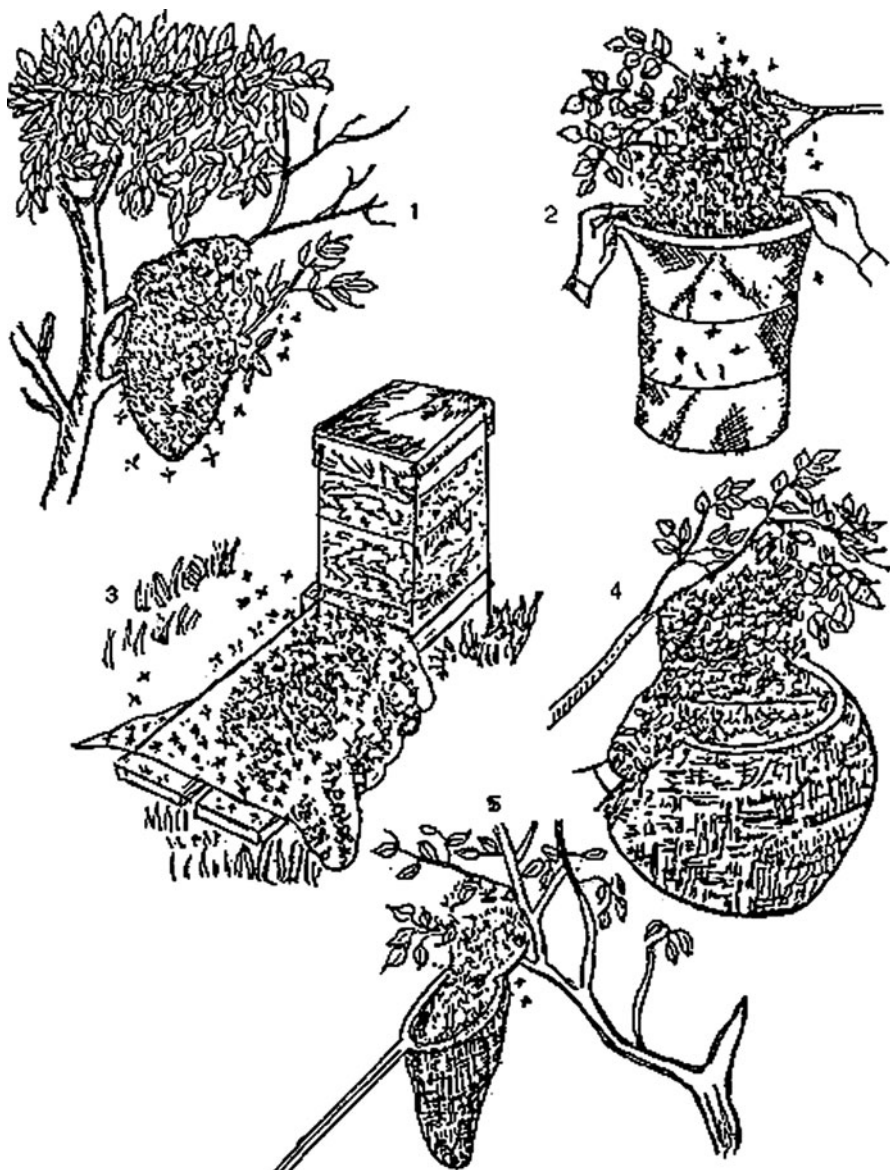
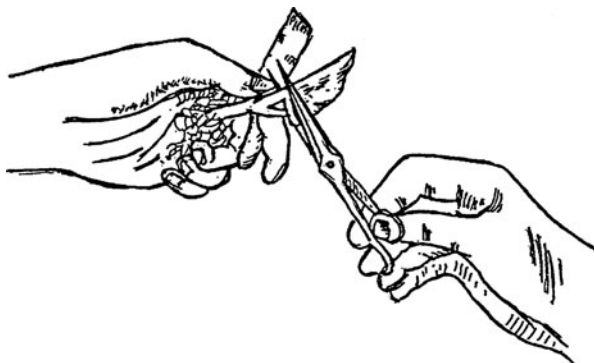


Fig. 15.7 Catching and hiving a swarm: 1 swarm on a tree, 2 capturing in a swarm bag, 3 hiving a swarm, 4 capturing swarm in a basket and 5 capturing swarm in a swarm catcher

proper ventilation, as lack of ventilation also enhances swarming. Remove one or two frames or brood (sealed), transfer it to another colony to provide additional space in the mother colony. Care should be taken that frames that are provided should have minimum number of drone cells or may have full foundation sheets only. The

Fig. 15.8 Clipping of the queen wings



production of more drones also results in issuance of swarms. All queen cells should be destroyed after every 4th day. When the brood chamber is almost full, place a super over it. A few brood frames along with workers can be removed from the colony and placed in the upper chamber or given to other weak colonies. In their place, empty frames may be provided. If a colony swarms in spite of the precautions, the swarm that issues may be caught and introduced into a new hive. Before the honey flow, this swarm can be reunited with the mother colony or with another weak colony. It has been observed that one strong colony will give twice the quantity of honey, as yielded by two weak colonies collectively having the same number of bees.

Swarming generally occurs in the forenoon between 10 AM and 11.45 AM, but occasionally it is noticed at other parts of the day. The bees come out in a rush and encircle the hive. When a fairly large number of bees have come out and the queen has accompanied, the swarm is issued. Other ways of prevention swarm is to use queen excluder which will allow the bees to pass, but not the queen or clip the wings of the queen (Fig. 15.8). If queen will not accompany, then no swarm will be issued. But, in a bee colony, if swarming impulse persists and is evident by raising regular queen cells, then best way is to divide the colony.

15.13.2.4 Management During Honey Flow

The honey now commences after the swarming fever is over. The seasonal onset of honey flow can be known from the whitening of honey and of the honey cells and from an increase in the weight of bee hives, exact period varies from locality and the bee keeper himself has to find out the precise time when the bees store surplus honey. Not knowing the period or slight delaying in catching up with the bees can deprive a beekeeper of the valuable gift of honey that can be extracted. During honey flow, the beekeeper must keep the honey gathering instinct of the bees in mind. The bees would thus require additional combs for the storage of the honey. When the brood chamber becomes full, the honey frames should be removed and placed in the upper chamber of the super. The main honey flow in the sub-temperate and temperate areas of the J&K state occurs during April, May or up to the middle of

June and in the higher hills in September and October. Depending upon the local climate, the altitude, the direction of the mountains, etc., the honey flow may be 1 or 2 weeks earlier or later and the beekeeper has to make adjustment likewise. In the subtropical areas, the honey flow may start as early as in the middle of March and may continue upto the middle of May, depending upon the locality.

15.13.2.5 Honey Extraction

The extracting of surplus honey from the colonies is the ultimate aim of beekeeping. It depends upon the efficiency of a beekeeper that how much surplus honey and how many times he can extract honey from a colony. The beekeeper must also know exactly when to extract honey and how much of it to leave as food for the bees. When the honey flow has begun to slow down or when it has just stopped, it is the best time to extract honey. The frames in which two-third or three-fourth of the cells have been scaled over are taken out of the hives, leaving behind 3–4 kg of honey in an Indian colony and approximately double the quantity in the Italian colony. The frames are removed in the evening and care is taken that there are no brood or bees on the frames. The frames are stored in a room in which bees cannot get in to rob honey and the extraction is done that very night.

The uncapping knife is heated in boiling water and with the sharp edge, wax-capping cut from the tops of the cells on both sides of the frames (Fig. 15.9). Two such frames are placed at a time in centrifugal honey extractor. The machine is revolved vigorously for about 2 min and the honey flows out of the cells. The other sides of the frame are then turned and the machine is worked again. As the honey flows out of the machine, it is strained through a piece of muslin. Honey is stored in stainless steel drums, tins or glass bottles. The container should be airtight to avoid the absorption of moisture from the air and the consequent fermentation of honey. The empty frames are returned to the hives and all the appliances used in honey extraction are thoroughly washed with water to avoid robbing and fighting among the bees. As a precaution, the bees entrances of all the colonies are shortened on the eve of honey extraction. Pure honey has the property of granulation after sometime, particularly during the winter season. If honey is heated in a water bath at 1,400 °F for half an hour and packed, it would not granulate so easily.

15.13.3 Summer Management

In most localities, honey-flow season is followed by the summer dearth period. Bees adopt number of strategies to protect their stores as they throw out the drones because they are useless for the colony. Unmanaged colonies stop brood rearing in order to not starve in future. The colony strength also declines considerably due to the death of old and decrepit bees who have put in strenuous work and exhausted due to honey gathering during the honey-flow season. In summer, besides defending the colonies against enemies, bees have to keep their hive cool and properly ventilated.

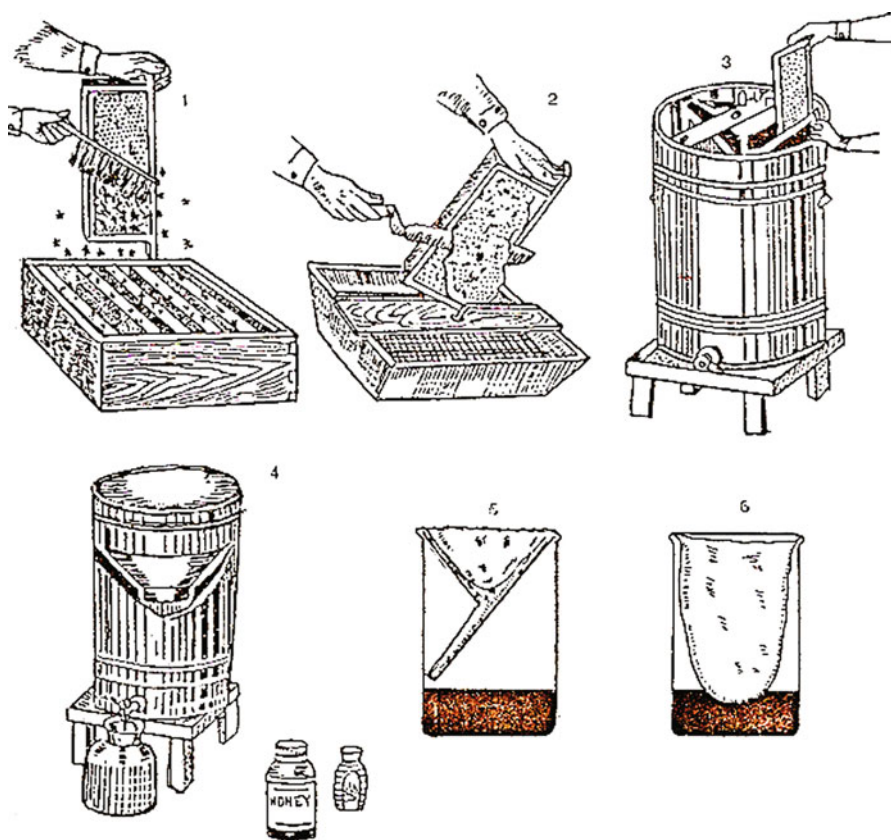


Fig. 15.9 Honey extraction: 1 brushing off the bees, 2 uncapping the cells, 3 putting frame into the extractor, 4 straining and bottling, 5 straining honey (correct method) and 6 straining honey (wrong method). (Redrawn from Rawat 1982)

In unattended colonies, the bees mostly remain indoors, do little work and wait for the developing brood to emerge. The queen stops laying eggs and if she persists in depositing eggs, they are neglected by the bees and allowed to shrivel. When almost all the brood has emerged and not enough is left, the colony absconds. Only infested combs and sometimes infested with wax moth are left. The deserting bees almost behave in a similar fashion as swarming bees except that these bees rise very high and it is rather difficult to make them settle down. Desertion or absconding is little known in *A. mellifera*, but the tendency is very strong in Indian honeybee *A. cerana indica*. *A. mellifera* colony would rather die because of starvation or due to attack of enemies, but show little tendency to swarm.

The best way to save colonies during this period is to avoid broodlessness in colonies and stimulate them to rear brood. The strong colonies with sufficient stores would continue to rear some brood during summer. Colonies headed by young prolific

queens will continue to lay eggs. Strong colonies are less troubled by enemies and are able to take advantage of subsistence floral sources. The colonies cannot be kept in the open sun.

The colonies should always be shifted to a place with thick shade. At some places, the temperature rises as high as 45–47 °C. Also, gunny bags, moistened twice at noon and in the afternoon, can be spread over top covers. Ventilation of hive is to be ensured, but holes, cracks and crevices also allow easy passage to enemies. The brood and super chamber can be raised slightly by putting shavings or splinters of wood in between. The space so made should not allow the bees to pass through to avoid robbing. Sufficient honey stores are must in a colony. Beekeepers should have complete information of subsistence flora that would be available to the bees and make his judgment about the stores to be allowed at a time of last honey extraction. The brood rearing in summer can also be boosted by feeding pollen substitute or pollen supplement, a continuous supply of which is required during dearth period since these are not stored in cells. Frequent inspection of colonies should be avoided. The above-mentioned management practices during summer check the absconding of colonies. Infact, all efforts should be made to add to the comforts of bees. Some of the other operations include:

1. Feed the bees with a thin sugar solution daily. One part of sugar and eight to ten parts of water. This also meets the water requirement of bees to maintain suitable humidity condition within the hive.
2. Make provision of fresh water daily.
3. Some sort of wind break should also be provided to the bees, so that the colonies are protected from the hot winds (Loo) of the day.
4. Keep the gunny bags or rice matting placed over the top covers of hives drenched with water to reduce the excessive temperature.

15.13.4 Monsoon Management

Honey-flow season is mostly over by the end of May and colonies are required to be prepared for monsoon management. For survival of the colony the first thing to be seen after honey-flow season or during the last extraction of honey is that adequate quantity of honey is left over in the brood and super combs for consumption by bees during the prolonged monsoon period of 2 months. The colonies should be well protected from rains, wind and enemies such as ants, lizards, wasps, birds and others. Mass desertions immediately after monsoon during September–October are a major problem for beekeeping. This is mainly due to the weakening of colonies, coupled with fungal growth, wax moth infestation on old combs uncovered by honeybees and general unhygienic conditions. Removal of empty combs at periodic intervals during monsoon saves colonies from wax moth to a greater extent. The major wet spell of monsoon is generally over by the mid or end of August and there are intermittent sunny days. At this time, weeds, grasses and many kharif crops such as coriander,

maize and oilseeds flower and bees start collecting pollen. Comparatively, nectar is not freely available. On a clear sunny day, the colonies should be quickly inspected and old black combs uncovered by bees or combs with fungus or wax moth infestation should be removed, even though such combs have some brood or stores. Wired frames fitted with comb foundation should be provided in place of the removed combs and 300–500 ml sugar syrup (50 % sugar by weight) may be given to the colonies in the evening. With availability of pollen and simulative sugar keeping, bees start constructing new combs. This may be repeated at 10–12 days intervals complete comb renewal can thus be done by end of September. Queen lays eggs III such newly drawn combs and there would be adequate sealed and unsealed broods in the colonies. With this management, the problem of post-monsoon desertions cannot only be avoided, but the colonies can be made strong for multiplication or minor honey production.

15.13.5 Post-Monsoon Management

Depending upon the duration of forage availability from October to December, severity of winter and the dearth of January, there can be two alternate methods that could be adopted.

1. If the honey-flow period is short, the colonies may be developed for minor honey flow. Some strong colonies may show signs of swarming. But, when the flow season is short it is not advisable to divide the colonies in view of the ensuing dearth periods. Sealed brood combs from such colonies may be removed to reduce their swarming impulse and given to comparatively weak colonies to make them strong as drawn out super combs, if available, may be given to the colonies at appropriate time. Alternatively, full comb foundation sheets may be fixed in super frames and these may be got drawn out for use during the major honey-flow season. After extraction of any surplus honey in the minor honey flow, the drawn out empty supers may be preserved using Paradichlorobenzene.
2. If post-monsoon flow is long, with sufficient pollen and nectar and if the winter rains are not heavy or winter dearth is not very acute, then few selected strong colonies may be strengthened further by giving them scaled brood frames and frames with pollen and honey stores from different colonies. This will induce early drone breeding and swarming impulse in them. Swarm cells from such colonies can be used either to increase the number of colonies or to requeen the colonies having old queens. In any case, it has to be seen that the divided colonies with newly mated queens get sufficient time in the latter part of the flow for their development and for building up pollen and honey stores for the following dearth period. Colonies headed by new queens over winter well.

15.14 Problem of Bee Pests and Diseases

15.14.1 *Bee Pests and Diseases*

The oriental honeybee has inhabited the Asian continent for millions of years, and over this long period various microbial diseases, parasites and predators have found ways of exploiting bee colonies. In fact, it is now believed that the bees' high rate of absconding is an inherited behaviour characteristic which has gradually evolved a response to pressure on colonies from heavy predation and parasitism. Pest control is one of the most important aspects of the management of *A. cerana*. While the primitive design of log and box hives does not allow for much choice in the way of pest-control measures, the least the traditional beekeeper can do is to ensure that the environment of his hives is free from major pests, and to make it difficult for pests to invade the hives. All cracks in hive walls should be sealed; the hive entrance should be kept as small as possible, to limit the ability of wasps and wax moths to enter the hive; to protect them from ant attacks, hives should be placed on stands whose legs are coated with grease or spent oil. But, colonies in simple hives cannot be manipulated effectively to control infestation of bees and brood within the hive by tree mites and microbial diseases.

15.14.2 *Bee Mites*

Two mite species are considered to be serious pests of *A. cerana* in Asia: the tracheal mite *Acarapis woodi* and the Varroa mite *Varroa jacobsoni*. For the most part, the other mites found in association with oriental honeybees feed exclusively on pollen, using the bees primarily as carriers. These "phoretic mites", particularly those belonging to the genus *Neocyphophthalmus*, may be present in large numbers in honeybee colonies, but apart from consuming stored pollen, and disturbing foragers when many of them hoard the bees at once, they are essentially harmless. A simple way of distinguishing between parasitic and phoretic mites is that the latter are not found within sealed brood cells, nor within the adult bees' tracheal system.

15.14.2.1 *The Tracheal Mite Acarapis woodi*

Beekeepers in parts of India and Pakistan have reported heavy losses in commercially operated colonies of *A. cerana* as a result of infestation by the tracheal mite. It is not yet clearly established whether the parasite was introduced into Asia via imported colonies of *A. mellifera* or whether it is indigenous to Asia. Whereas some beekeepers and apiculturists suggest that *A. cerana* colonies are more susceptible to the tracheal mite than colonies of the European honeybee, others maintain that the reported losses of colonies are due mainly to the spread of *Apis* iridescent virus (see Sect. 15.14.3) or to a combined attack by the mite and the virus.

15.14.2.2 Symptoms and Diagnostic Procedure

It is difficult to determine the presence of tracheal mites in honeybee colonies: the parasites are very small, and they infest the host bees internally, in the thoracic trachea. Adult bees infested by *A. woodi* show no noticeable signs, but their life-span is shorter than that of uninfested bees; as a result, rapid decreases in colony populations in winter and spring can be observed. The only reliable diagnostic method is the microscopic examination of dissected tracheae of sample bees from colonies suspected of being infested. If present, the mites are usually found within the trachea closest to the bees' thoracic spiracles; the infested tracheae display a colour darker than normal.

15.14.2.3 The Ectoparasitic Bee Mite *Varroa jacobsoni*

The bee mite *Varroa jacobsoni* is a parasite of *A. cerana* indigenous to the entire continent of Asia. Wherever colonies of the oriental honeybees are kept, there is therefore a possibility of mite infestation. Through millions of years of being parasitized by the mite, the bees appear to have developed some degree of resistance to its attacks. Abscinding is one of the colony's manners of ridding itself of the mite, or at least of those infesting the brood, which in such cases is abandoned. Colonies heavily infested by *V. jacobsoni* produce little or no honey, but most often the beekeeper can lose the entire colony when it absconds.

15.14.3 Viral Diseases

At least three viral diseases affecting *A. cerana* are known: the Thai strain of sacbrood virus (TSBV), the Kashmir bee virus (KBV) and *Apis* iridescent virus (AIV). While TSBV affects the brood, the latter two diseases affect the adult bees. TSBV was first reported in *A. cerana* colonies in Thailand and has since been reported from other Asian countries. Its natural distribution range may cover the entire Asian continent where feral colonies of the bee exist. In Thailand, the disease is found in colonies under "stress" conditions: lack of food, excessive humidity, low worker population, poor-laying queens, etc. Little is known about KBV, except that it has also been reported in *A. mellifera* colonies in Australia. In its first recorded presence, It was identified together with AIV in bees from Kashmir. Recent reports state that AIV has been causing serious damage to commercial colonies of *A. cerana* in northern India and Pakistan, the virus being associated with "clustering disease". The bees are unusually inactive; they frequently form small, detached clusters of bees that do not fly. Many individual bees are observed crawling on the ground and are lost. At first, these symptoms were associated with the presence of the tracheal mite *A. woodi* on some diseased bees, but it was later shown that AIV is the major causative agent.

15.14.3.1 Treatment

No chemical treatment that can be used effectively against the viral diseases of honeybees is available. Since it has been observed that the diseases are most frequently found in colonies under stress conditions, it appears that strengthening the colonies and providing better hive environments are among useful preventive measures. It is suggested that among measures to be taken are re-queening the colonies with young, healthy queens, supplemental feeding, adding frames of older brood, and protection of hives from cold, humidity and strong wind.

15.14.4 Microbial Diseases

Colonies of *A. cerana* are occasionally found infested with bacterial diseases such as American foul-brood and European foul-brood; other microbial diseases have also been reported. Since much of the technical information concerning the microbial diseases of *A. cerana* is based on that obtained from experience with *A. mellifera*, recommendations for their prevention and control with respect to the latter species are also applicable to beekeeping with *A. cerana*.

15.14.5 Abscending

Though *A. cerana* are poor honey yielder, they require low management costs, are efficient pollinators and adopted on the harsh mountain environments including pests, diseases and predators in mid-hills. However, this bee is aggressive, swarms frequently and absconds hindering the commercial bee keeping (Tokuda 1924, 1935; Roepke 1930; Kellog 1941; Sakagami 1960a, b). In fact, honeybees are known to possess intricate sequence of behaviour through which they monitor their environment. When the nest ecology deviates from normal and the situation inside is deteriorated, they react immediately. At extreme situations, the whole members of the colony abscond (Kafle 1985). Non-availability of food and water (Smith 1961; Fletcher 1978; Sakagami 1960a; Singh 2000; Hassan 2003), abnormal climatic conditions (Ruttner 1988), poor ventilation, attack of diseases, pests and robbing, hazardous fumes, pesticide poisoning (Singh 2000), presence of old combs (Kapil 1960; Verma 1970; Ruttner 1987), physical disturbances and unusual handling (Singh 2000) are the causes of absconding in different parts of the Asian tropics. Pokhrel et al. (2006) found that in 12 colonies of *A. cerana* studied, one-third of the colonies absconded in summer and about one-sixth in rainy season, while non-absconded colonies also slowed comb building, brood rearing, colony strength and hive storage in summer and rainy seasons. Feeding sugar candy and pollen substitute prevented absconding in May and July. Three weeks feeding in May resulted higher comb building (15.0 %), higher brood rearing (158.8 %), stronger colony strength (15.0 %) and higher hive

storage (171.2 % honey, 270.9 % pollen) in June. Those colonies having higher brood mite (*Varroa jacobsoni* Oud.) in winter absconded earlier.

The suggested ways to prevent absconding are: timely examining bee enemies and diseases and their management, and feeding artificial diet or putting queen gate at hive entrance, etc. Koeniger (1976) recommended transporting bee colonies to pasture or intensive feeding during dearth and adopt proper seasonal management practices. Sugar syrup feeding during off-season (rainy, summer and winter) prevented absconding of *A. cerana* (Verma 1984; Sakagami 1960a; Smith 1961; Fletcher 1978; Singh 2000).

During the course of evolution, *A. cerana* has developed certain behavioural characteristics such as frequent absconding and swarming which are essential for the survival of colonies, but undesirable from beekeeping point of view. It is one of the most disappointing things that can happen to a beekeeper in a developing country is to have a absconded colony. It is not only disappointing, but may seriously affect the viability of the beekeeping business, so some understanding of the factors that underpin absconding are important to beekeepers. Absconding may be defined as the complete abandonment of the nest by the whole colony. It differs from swarming in that the nest does not divide into two or more parts, but the whole colony moves and presumably seeks and finds a new nest site elsewhere. The lack of sufficient bee flora, excessive handling, exposure of colonies to summer sunshine and incidence of sacbrood virus disease may be the major causes of absconding. Colony performance index (CPI) reaches zero even a week before absconding. Management practices such as feeding sugar, provision of shade, providing queen gate at the hive entrance significantly reduce absconding. However, colonies affected with sacbrood virus disease show such a severe instinct of absconding that these may leave the hive even without queen bee. The tendency to abscond is mainly determined by climate and the effects of climatic change on flowering and nectar flow. Honey bees have two possible strategies for surviving periods of dearth: (1) by storing sufficient quantities of honey to enable them to survive during the dearth period or (2) by moving to another place where there may be greater nectar flow available.

One of the most effective way of reducing frequent swarming is to follow selection programme against this undesirable trait and removal of newly constructed queen cells during active swarming season also help to check it considerably. Currently, recurrence of sacbrood virus epidemic after an earlier cycle during 1982–1986 has threatened beekeeping with *A. cerana* throughout its range and is forcing beekeepers for its replacement by more profile *A. mellifera*. Some colonies are still resistant to this disease and in the absence of any effective chemical control measures, vigorous selection programme need to be followed (Verma 1992). The problem of absconding, which has always been difficult for the beekeepers of *A. cerana*, is mitigated if the beekeeper keeps the hives fed with sugar and pollen, during the off-season. In addition, bees can be found in the jungle and the swarms can easily be brought back to the apiary for transfer into the frame hive.

For temperate bees, the absconding strategy is almost never used as it is likely to be fatal to the colony—an absconded colony would be most unlikely to collect enough stores for it to survive the lengthy dearth periods it will encounter. However, tropical

species of *A. cerana* may utilise either the hoarding or the absconding strategy to survive dearth periods and may show high levels of absconding. There are two types of absconding: planned and unplanned. Winston (1987) calls this disturbance-induced absconding and resource-induced absconding, respectively. These definitions speak for themselves; disturbance-induced (or unplanned) absconding can be induced by predation, or invasion of the colony by undesirable pests such as hive beetles, ants or wax moths. Poor physical conditions such as entry of water into the hive, excessively high temperatures due to lack of shade or shortage of water, the proximity of bush fires or excessive disturbance can also encourage colonies to abscond. Resource-induced (or planned) absconding appears to occur due to scarcity of nectar, pollen or water and occurs primarily during the dearth periods found in tropical conditions. Resource-induced absconding tends to be seasonal and differs from disturbance-induced absconding in that colonies start preparing to abscond up to 1 month in advance of actually leaving. Firstly, the queen will reduce her egg-laying rate, so that very few larvae will be reared during this period. Those few eggs that are laid will be eaten by the workers. Most of the stored pollen and honey is also consumed by the bees. As soon as the last young brood has emerged the colony will abscond. By doing this they ensure they have a good number of relatively young bees and the consumption of pollen means their fat bodies will be full of stored protein, ready to start rearing new workers in the new place.

Absconding rates are on average 15–30 % annually and can be as high as 100 % in some seasons. These are important factors to take into account. In essence, it means that it is likely that up to one-third of the bees in any one place can be lost. The beekeeper needs to take this into account when calculating the number of hives that need to be kept if beekeeping is being undertaken as a business rather than as opportunistic honey collecting. It is especially important to consider if a high-cost system of beehives had been taken on, especially if this has started with microfinance borrowing.

Since not all colonies abscond, these factors are not the complete answers to the questions posed by absconding. One feature of absconding is the condition of the colony as well as the availability of resources. Winston (1987) notes that colonies that abscond are those that have swarmed within 6 weeks of the dearth period which would leave them with fewer workers, more older workers and a lower brood viability than colonies that have not swarmed. He also notes that absconding colonies can travel much greater distances than normal swarms—up to 160 km before starting a new nest site. They may travel through areas of poor resources, sending out scout bees to search for suitable forage until they find an area of greater resources to settle in. Where it is possible, feeding bees can help to limit the level of absconding, but it would take good observation and assessment of the colonies that are likely to swarm to make this worthwhile. Aggressive predation and resource limitation in the tropics is likely to lead to higher death rates for topical colonies. However, short intervals between swarming and high number of swarms are likely to offset higher death rates among tropical bees.

Reproductive swarming, absconding and migration, depends upon the extent of resource investment ascribable to climate and the seasonality of flowering in tropical and temperate regions (Koeniger and Koeniger 1980; Seeley 1985; Ruttner 1988;

Nakamura 1993, 1995; Hepburn and Radloff 1995, 1998). These are the essential prerequisites that both allow and drive honeybees to abandon their maternal nests and to establish new ones. Temperate-zone honeybees have only brief periods of weather favourable for swarming and establishing new colonies and nests and conserve large stores to survive winter; few abscond or migrate (Martin 1963). In sharp contrast, tropical climates with perennial flowering facilitates swarming, absconding and migration (Seeley 1985; Nakamura 1993, 1995; Hepburn and Radloff 1995, 1998). However, predation pressure is severe in many tropical areas and must also select for greater colony mobility through absconding and migration. Absconding/migration may be beneficial to the survival, dispersal and propagation of some honeybee species, but imposes serious difficulties in beekeeping in the tropics (Smith 1961; Crane 1990; Soares and De Jong 1992; Verma 1992). This has stimulated observations and experiments over the last two centuries that are beginning to provide a biological basis for absconding, migration and reproductive swarming in the context of insect sociality.

Absconding is also the movement of a whole colony away from its maternal nest site to another place. The inferred causes of absconding are chronic disturbance and/or predation, declining quality of the nest site, and resource depletion. Absconding is reflected in the behaviour of a colony preparing to abandon its combs.

15.14.6 Seasonality of Prepared Absconding and Migration

The climatic conditions that lead to seasonal dearth and then to migration obviously vary. *A. cerana* migrates during periods of high temperatures and again during the dearth of the dry season (Dulta et al. 1988), or follows the abatement of the heavy rains (Olsson 1989). A complex pattern occurs in the mountains in Sichuan Province, China where most colonies migrate/abscond every year. The region is a highly convoluted landscape where subtropical and temperate climatic regimes converge. So, some species of flowers are in bloom for a long time, but in different places, so that *A. cerana* migrates continually (Chen 1995).

The obvious effects of resource depletion can be directly measured as changes in honeybee colonies. Dulta et al. (1988) fortnightly recorded the demographic changes in ten strong and ten weak colonies of *A. cerana*. In all of the weaker colonies which absconded/migrated, honey and pollen stores, eggs and open and sealed brood decreased by nearly 90 %. In contrast, the ten stronger colonies, which did not abscond had increased honey stores and had only small decreases in pollen stores and brood area.

The interpretations as to causes of absconding/migrating are supported by experiments to avert it. Woyke (1978) gave brood and pollen to a colony of *A. cerana* and over the next 2 days the bees ate all of the brood, but did not forage for pollen. When additional combs of brood of all ages were supplied, they began to forage, rear brood and absconding was averted. During a poor pollen flow, the queens continue to lay resulting in more larvae than nurse bees can feed. The workers eat older larvae to

produce bee milk to feed the queen and other larvae. Other attempts to prevent absconding/migration by supplementary feeding and manipulation of the combs were also successful (Olsson 1989; Wei 1994; Lin 1977).

Resource-related absconding/migration is a strategy to overcome long periods of dearth while cannibalism is adapted to overcome shorter periods of dearth or small fluctuations in forage resources (Nakamura 1993, 1995). The rationale is that a honeybee colony has several metabolic buffers to reduce the effects of fluctuation in the normal field resources: pollen stocks, the secretions of the hypopharyngeal glands of the workers and brood which can be recycled through cannibalism.

The role of pollen in prepared absconding or migration by *A. cerana* was quantitatively investigated by Punchihewa et al. (1990) in field studies in Sri Lanka. Similar to Woyke (1978), they noted that one of the distinct characteristics of a colony preparing to abscond is a decline in the numbers of returning foragers carrying pollen. The preparative stages are phasic and gradually build up and can be measured as the progressive reduction in pollen foragers over time. Punchihewa et al. (1990) developed what they termed a “colony performance index” defined as the total number of returning foragers bearing pollen loads divided by the periods of observations (in seconds) multiplied by the total number of returning foragers. In the course of preparative absconding, the index gradually falls to 0.00 on the actual day of absconding. These authors also noted that once the absconding impulse is activated, the bees no longer defend the nest against intruders such as wax moths and ants and cease to rear brood.

15.14.7 Predation Pressure

Pests and predators as well as robbing among colonies of *A. cerana* are generally regarded as major contributory factors for prepared absconding throughout China (Wei 1994; Chen 1995). Olsson (1989) demonstrated the impact of pests and predators by narrowing hive entrances to restrict the entry of wasps and beetles. Reduction in the numbers of combs and replacement of old with new combs also reduces the chance of wax moths gaining a deleterious foothold in the nest. Likewise, uniting all weak colonies greatly reduced absconding, in part, by providing a stronger defence force against natural enemies.

15.14.8 Microenvironmental Effects

Absconding intensity by *A. cerana* is closely related to ecological/climatological conditions: it is most intense in dry lowland areas and becomes less acute in wetter areas and with increasing altitude in Sri Lanka (Punchihewa et al. 1990). Environmental effects are particularly evident where different climatic systems converge. So, in Sichuan, China where very weak colonies cannot effectively thermoregulate, they normally abscond and often amalgamate with others (Chen 1995). More

direct effects of microclimate on the absconding impulse is seen in colonies of *A. cerana* kept in hives or natural nest cavities. All of the hived colonies attempted to abscond while none in natural nest cavities did so (Punchihewa et al. 1990). The difference was attributed to micro-climatic conditions in the hives. The evidence for this conclusion was that a reduced “colony performance index” in hived bees could be experimentally increased by control of temperature and humidity regulation in hives. This has the effect of averting a low index, which activates the absconding impulse. Moreover, Punchihewa et al. (1990) were able to artificially reduce the index of a colony by constant disturbance, smoke and high temperatures.

15.14.9 Absconding/Migration Behaviour

Koeniger and Koeniger (1980) demonstrated that a modified dance language is associated with absconding/migrating of bees. They recorded the migration dances of *Apis dorsata*, which entail only waggle dances of very long duration. The bees do not return to their starting point at the end of a run but begin anew elsewhere. They do not complete the total cycle of the usual waggle dance, thus separating a migration dance from the waggle dance associated with foraging. Essentially, the same observations were made for absconding colonies of *Apis florea* (Free 1977; Dyer and Seeley 1994) and *Apis mellifera scutellata* (Schneider and McNally 1994) and *A. cerana* (Sasaki 1991).

Desertion of the nest and successful re-establishment elsewhere requires that the bees have sufficient flight fuel and reserves of energy to construct new combs when they have settled. Engorgement is a normal step in preparing to abscond/migrate in *A. m. scutellata* (Otis et al. 1981) as well as for *A. cerana* in China (Chen 1995). Reduction in egg laying commonly precedes migration and so conserves protein. Likewise, the worker bees commonly eat even sealed brood in the colony before leaving the nest. Thus, full preparation for migration includes elevated wax production, increased protein uptake and massive fuel intake. In a breeding programme using recurrent selection, the tendency for both swarming and absconding could be reduced in *A. cerana* (Deodikar and Thakar 1966) and in *A. mellifera* (Foti 1980).

The genetic variability for the “prepared absconding” trait is evidenced by honeybee reaction to the seasonal nature of pollen-income cycles, which start and stop during any particular season. Parallel observations were that some colonies preparing to abscond did not collect pollen or rear brood even if pollen-flow conditions improved in the area. Yet, other colonies in the same apiary quickly responded to available pollen and reached peak development. So, after such a pollen flow diminished, some colonies immediately ceased brood rearing and prepared to abscond, others not. These constitute significant and probably genetically based differences among *A. cerana* colonies exposed to the same environmental conditions in the same apiary (Woyke 1976; Nakamura 1993, 1994, 1995). The significance of resource depletion as the general driving force for large-scale migratory movements is also reflected in *A. cerana* (Ahmad 1989; Nakamura 1993).

15.14.9.1 Reducing the Likelihood of Absconding

It will be recalled that absconding is the colony's natural response to such unfavourable hive environments as lack of food or attacks by the bees' enemies. Correcting these situations can to some extent deter the colony from absconding. In marginal foraging areas, where food is not abundant all year round, supplementary feeding during the dearth period is necessary, especially, as already noted, when all or most of the stored honey has been harvested. Colonies of *A. cerana* are highly responsive to threats by the bees' natural enemies, and it is of the utmost importance for the beekeeper to make every effort to protect his colonies against attacks by bee pests. Heavy predation by hornets, ant attacks, wax moth infestation, and parasitism by bee mites are among the major problems to be dealt with. In this regard, the techniques indicated in Chap. 6. for the protection of *A. mellifera* colonies apply to *A. cerana* colonies as well. It has often been suggested that a mass programme to select *A. cerana* races for reduced absconding behaviour constitutes a priority sector in an apicultural development programme. From the practical standpoint, however, genetic manipulations of honeybees are usually difficult. The queens' multiple mating with drones in mid-air forms an obstacle to maintaining any particular breed or gene pool. Drones from feral nests are a major hindrance to selective breeding. Unless the breeding site can be isolated, it is impossible to ensure that queens of the selected stocks mate with drones of the breeder's choice, so that the genetic quality of the offspring cannot be guaranteed. *A. cerana* queens can be artificially inseminated, but the technique is difficult even under normal laboratory conditions, and transferring this technology to rural beekeepers would create many problems. Further, should the selection approach be adopted, a special queen-breeding station for large-scale distribution would be required. It thus appears that the most practical, and therefore the most appropriate approach thus far available for minimizing the absconding behaviour of *A. cerana* colonies lies in good colony management such as good hive construction, suitable apiary sites, supplemental feeding during dearth periods, prevention and control of honeybee pests and diseases, etc.

15.14.10 Control Over Desertion of Bee Colonies

Desertion is one of the problems faced by beekeepers. During spring period, nectar and pollen are available to the bees in abundance and swarming and colony multiplication takes place during this period. After the spring, the major honey-flow season commences which is followed by a prolonged dearth period and adverse environmental conditions for the bee colonies. This results in desertion of a large number of colonies. Desertions occur mainly during monsoon or post-monsoon period, i.e. in August–September or in the beginning of October. This particularly occurs in areas where spring and summer are building up and honey-flow periods and summer monsoon is prolonged dearth period.

In other regions, where September–October is a flow period, desertions occur in winter or during winter rains. The losses are to the tune of 40 % and go upto 60 % or

more, particularly when build-up period or honey-flow season fails. In either case, about 50 % of the bee colonies desert and the remaining 50 % of the colonies though surviving the dearth are in a very weak condition. These colonies take a long time to develop and to come to swarming stage or to honey-yielding stage. As a result of this situation, the colony number in the country remains almost static and at times honey production goes down. It is therefore necessary to know fully the reason for desertion or dwindling of colonies and the precautionary measures necessary to avoid the colony losses due to desertion. The main causes of desertion or dwindling are as follows: starvation, unhygienic conditions in colonies, absence of young bees in colony and high temperature and humidity.

15.14.10.1 Starvation

In the hills, where most of the beekeeping activity is concentrated, high rainfall and foggy conditions confine the bees to the nest for at least 2 months. So, adequate stores of honey are not left in the colony by the end of honey-flow season, the bees starve and the colony perishes. The surviving colonies try to desert during sunny days of dearth period.

15.14.10.2 Unhygienic Conditions in Colonies

Most of the brood combs used for brood rearing during spring period get old and black. After the emergence of bees, these combs are not used as the queen stops laying eggs after flow. Due to depletion in bee population, such combs are not covered by the bees. Such old black brood combs generally get heavily infested with wax moth and fungus. As the strength of the colony dwindles, other pests, predators and enemies of bees such as wasps, ticks, mites, ants, birds, etc. further demoralize the bee colony. This also results in robbing and fighting among bee colonies and finally the colonies attempt to desert en masse.

15.14.10.3 Absence of Young Bees in Colony

At most of the places, flow period is followed by dearth period. By the end of this period queen stops laying eggs. Having worked vigorously, most of the worker bees get old, worn out and exhausted. These are of very little use after 2–4 months long dearth period.

15.14.10.4 High Temperature and Humidity

Water management of bee colonies is a very important factor. During summer, requirement of water of the bee colonies increases. Large number of bees collect

water for maintaining the temperature of the colony and for brood rearing. If fresh water is not available near the apiary, working of the colony is affected. Bees cannot tolerate excessive humidity and accumulation of water in the colony. This leads to unhygienic condition in colony and prevalence of brood diseases.

Desertions can be avoided to a great extent by proper management of colonies during pre-monsoon and post-monsoon period or during flow and winter dearth period. The following precautions may be taken to prevent desertion:

1. Leave adequate quantity of honey stores in the colony. Do not extract all the honey flow. This help in two ways: adequate stores are available for the prolonged dearth and bees are also not overstrained.
2. During building up and flow period, get two or three newly drawn combs by giving comb-foundation strips.
3. Ensure that all the colonies are headed by young healthy queens of less than 2 years of age.
4. Ensure that egg laying of the queen continues as late as possible in the flow period. This will ensure enough force of young bees, emerging from brood during dearth period, and which will be useful as foragers after the dearth period.
5. Keep queen gate in position and undertake proper repairs of the bee hives if required to protect the bee colonies from the attack of wasps, reptiles, etc.
6. The colonies may be kept in shade for protection from direct rains, dusty winds. Avoid accumulation of water on floor board by keeping the colony in a slightly inclined position.
7. Clean and dry the bottom board periodically. If possible use reversible bottom board. This may be useful to check the attack of wax moth in colonies.
8. In the month of August or when the severity of monsoon is reduced, inspect the colony casually on a clear sunny day; remove old black and uncovered combs and give dilute (50:50) simulative feeding.
9. A colony showing symptoms of desertion such as dull foraging, no sealed stores, no larval brood, etc. may not be kept in the apiary. It may be kept in isolation to avoid nuisance to other settled colonies in the apiary. When one or two such attempt desertion, generally bees from other good colonies also joins the deserting bee colonies, get mixed up and fighting starts, demoralizing settled colonies.
10. When the severity of monsoon reduces, bees may start collecting pollen. If the rate of pollen collection increases, give frames fitted with comb-foundation strips and heavy dose of sugar feeding. Continue this management if the bees have drawn out of the strips and the queen has started laying eggs, till all the old black and uncovered combs are removed.

The above-mentioned management practices will protect the colonies from pests and predators and induce bees to build new combs and collect pollen, have freshly drawn-out good combs in the colony for the queen to lay eggs and have fresh young bees to take advantage of the post-monsoon/post-winter flow period. This will not

only prevent desertions, but will also develop the colonies to multiplication or honey-yielding stage.

Another way to prevent desertion is to migrate bee colonies to comparatively less rainfall area. Areas having rainfall between 600 and 1,200 mm are ideally suited for monsoon migration. These areas have arboreal, ground and agricultural plants and many a time not only survival but multiplication of colonies or honey extraction is possible. If the forage is available for a prolonged period, queen cells can be used for re-queening and removal of old queens or for increasing the colony number. By the end of October or mid-November or by the end of February or early March, strong and healthy colonies can be migrated back to their permanent locations.

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Chapter 16

Population Decline

16.1 Introduction

Concerns have been raised that invertebrate pollinators of crops and wild plants are in decline as a result of modern agricultural practices, habitat degradation, and introduced pests and diseases. This has led to demands for a response by land managers, conservationists, and political decision makers to the impending “global pollinator crisis.” Until very recently, most farmers considered pollination as one of nature’s many “free services,” so taken for granted that it has rarely figured as an “agricultural input” or even as a subject in agricultural science courses (FAO). This assumption has apparently become obsolete as these changes are already being illustrated, need to be monitored and mitigated in the near future, posing threats to the integrity of biodiversity, global food webs, and even ultimately to human health.

The Food and Agriculture Organization (FAO) of the United Nations (Williams 1996) estimates that among slightly more than 100 crop species that provide 90 % of food supplies for 146 countries, 71 are bee-pollinated (mainly by wild bees), and several others are pollinated by thrips, wasps, flies, beetles, moths, and other insects. In Europe alone, 84 % of the 264 crop species are animal-pollinated and 4,000 vegetable species have their life assured, thanks to the pollination of the bees (Ingram et al. 1996). Pollinators are essential for the reproduction of many wild flowers and crops: for one out of every three bites eaten, one can thank a bee, butterfly, bat, bird, or other pollinator (Free 1993). The economic value of pollination worldwide is thought to be between ≤ 30 and 70 billion each year (i.e., € 45–100 billions). Any loss in biodiversity is a matter of public concern, but losses of pollinating insects may be particularly troublesome because of the potential effects on plant reproduction and hence on food supply security. Many agricultural crops and natural plant populations are dependent on pollination and often on the services provided by wild and unmanaged pollinator communities (Cane and Tepedino 2001).

16.2 Pollinators: An Essential Component for Ecosystem Functioning

Crop-associated biodiversity (CAB) refers to biodiversity that supports the functioning of ecosystem services necessary for agriculture, as well as contributing to the maintenance of ecosystem health and resilience. CAB is an intrinsic and important part of agricultural ecosystems and includes components such as pollinators. Pollinators contribute to the maintenance of biodiversity and ensure the survival of plant species including plants that provide food security to innumerable rural households. Pollination is an essential ecosystem service that enables plant reproduction and food production for humans and animals (fruits and seeds also impact on the quality and yield) that depend, to a large extent, on the symbiosis between species, i.e., the pollinated and the pollinator. The reduction and/or loss of either will affect the survival of both.

Pollinator diversity is directly dependent on plant diversity and vice versa—no other natural phenomenon illustrates more vividly the principle that conservation measures must be directed at ecological processes, and not just individual species. Indeed, pollination, a fundamental step for plant reproduction, is an ecological service that cannot be taken for granted. Plants are the primary producers in terrestrial ecosystems and direct providers of many ecosystem services such as carbon sequestration, prevention of soil erosion, nitrogen fixation, maintenance of water tables, greenhouse gas absorption, and food and habitat providers for most other terrestrial and many aquatic life forms. Pollinators, through facilitating plant reproduction, thus play a crucial role in the maintenance of ecosystem services. Pollination requires pollinating agents, which themselves require resources for nesting, feeding, and reproduction in the form of vegetation, prey, and certain habitat conditions, as well as the application of pollinator-friendly land-use management practices to ensuring their survival.

16.3 The Pollinators

More than 75 % of the major world crops and 80 % of all flowering plant species rely on animal pollinators (Nabhan and Buchmann 1997; Kevan and Imperatriz-Fonseca 2002). Of the 100 or so animal-pollinated crops, which make up most of the world's food supply, 15 % are pollinated by domestic bees, while at least 80 % are pollinated by wild bee species and other wildlife (Prescott-Allen and Prescott-Allen 1990; Ingram et al. 1996). Diversity among species, including agricultural crops, depends on animal pollination. Thus, pollinators are essential for diet diversity, biodiversity, and the maintenance of natural resources.

The principle pollinators are bees. Approximately, 73 % of the world's cultivated crops, such as cashews, squash, mangoes, cocoa, cranberries, and blueberries, are pollinated by some variety of bees, 19 % by flies, 6.5 % by bats, 5 % by wasps, 5 % by beetles, 4 % by birds, and 4 % by butterflies and moths (Fact Sheet: Pollinator

Diversity 2004). Of the 100 principal crops that make up most of the world's food supply, only 15 % are pollinated by domestic bees (mostly honeybees, bumble bees, and alfalfa leafcutter bees), while at least 80 % are pollinated by wild bees and other wildlife (as there are an estimated 25,000 bee species, the total number of pollinators probably exceeds 40,000 species).

The 25,000 different species of bees differ significantly in size and habit requirements and diverge accordingly in the plants they visit and pollinate. Though bees form the most important group of pollinators, other animals such as bats, birds, butterflies, moths, flies, and beetles also play key roles in pollination. Both the diversity of wild plants and the variability of food crops depend on this diversity.

Pollination is a complicated process with some pollinators being generalists and others being species specific. Similarly, many different pollinators visit some plants, while other plants have species-specific pollinator requirements. Given this complexity, managing pollination as an ecosystem service requires a comprehensive understanding of the pollination process and the application of that knowledge in the design and implementation of intricate management practices. In most cases, there is limited knowledge about the exact relations between individual plant species and their pollinator

16.4 The Importance of Bees for Pollination

No other group of insects are of more benefit to humans than bees. More than one-third of the world's crops require pollination to set seeds and fruits, and most meat and dairy industries rely on bees for pollination of clover and Lucerne (Dias et al. 1999). Crops relying on bee pollination include apple, citrus, tomato, melon, strawberry, apricot, peach, cherry, mango, grape, olive, carrot, potato, onion, pumpkin, bean, cucumber, sunflower, various nuts, a range of herbs, cotton, alfalfa, and lavender. The annual value of this service is estimated at US\$ 112 billion worldwide (Southwick and Southwick 1992). Even crops that do not require pollination for harvesting, such as those producing fibre or timber, still require pollination to produce further generations, and crops such as cotton that do not require pollination to produce seeds, provide greater yields when pollinators are available (Allen-Wardell et al. 1998). The European honeybee (*Apis mellifera*) dominates crop pollination worldwide, but local native bee species also play their part.

The population of both wild and managed pollinators is declining at alarming rates owing to alteration in their food and nesting habitats, shrinkage in natural ecosystems, i.e., forests and grassland ecosystems, pesticide poisoning, diseases and pests, overcollecting, smuggling, and trading in certain rare and endangered species. Insects (butterflies, moths, bees, wasps, ants, beetles, etc.) numbering about 500 species are an important supplementary source of calories and proteins in many regions of the world. Honeybees, economically the most important crop pollinator worldwide, are in a declining state. The number of commercial US bee colonies had

fallen from 5.9 million in the late 1940s to 2.7 million in 1995. The loss of one-quarter of all managed honeybee colonies since 1990 signals one of the most severe declines US agriculture has ever experienced in such a short period. An estimated 20 % of all losses of honeybee colonies involve some degree of pesticide exposure. Some pesticides, highly toxic to bees and birds are: aldrin, carbaryl, carbofuran, diazinon, dieldrin, endosulfan, EPN, fenthion, heptachlor, malathion, monocrotophos, parathion, phosmet, etc. In a recent field study at Cornell University in the United States, it was found that monarch butterfly caterpillars eating *Bacillus thuringiensis* (Bt) corn toxic pollen blown on to milkweed plants near Bt corn fields had suffered significant adverse effects leading to death of nearly 20 % of the caterpillars. These chemicals and toxins can eliminate nectar sources for pollination, destroy or adversely affect larval host plants for moths and butterflies, and deplete nesting materials for bees. Gardeners, orchard growers, farmers, and urban dwellers can switch to more pollinator-friendly organic methods of cultivation to reduce wildlife exposures to insecticides, herbicides, and fungicides.

About 75 % of genetic diversity of agricultural crops lost since the beginning of twentieth century from the earth and 25 % of the world species present in the mid-1980 will be lost by 2015 (FAO 1993). More than 85 % of the 7,000 or so apple variety grown in last century is now extinct in the United States (FAO 1993). In 1970, genetic uniformity of maize in the United States caused almost \$ 1,000 million loss and yield reduced by as much as 50 % (Thapa 2006). Wilson (1988) estimated that 0.2–0.3 % of all species are lost every year.

Animal-mediated pollination contributes to the sexual reproduction of more than 90 % of the approximately 250,000 species of modern angiosperms (Kearns et al. 1998). This interaction diffusely affects human survival through its roles in sustaining much biodiversity on Earth and contributing to the integrity of most terrestrial ecosystems. However, we also depend more directly on this interaction, because many agricultural crops rely to some degree on pollinators for setting the seeds or fruits that we consume, or the seeds we sow or breed. A now well-known estimate proposed that about one-third of our food, including animal products, derives from animal-pollinated, mostly bee-pollinated, crops (McGregor 1976). This estimate has recently been confirmed by Klein et al. (2007), although animal production was excluded. The diversity of crops that depend on animal pollination provides still more impressive estimates. For instance, biotic pollination improves the fruit or seed quality or quantity of about 70 % of 1,330 tropical crops (Roubik 1995) and 85 % of 264 crops cultivated in Europe (Williams 1994). These figures are not obviously biased by the inclusion of many minor crops from a production viewpoint, as pollinating insects increase fruit or seed quality or quantity of 39 of the 57 major crops world-wide (Klein et al. 2007). Therefore, the production and diversity of agriculture seem to depend to a large extent on biotic pollination, particularly on the service provided by the honeybee (*A. mellifera*), the single most important pollinator species, and a plethora of wild bee species.

There are more than 1,500 species of butterflies in the Indian subcontinent, but their population is dwindling because of the indiscriminate use of insecticides and chemical weed killers as well as atmospheric pollution. Many other man-made environmental changes such as deforestation, extension of farming, and unrestricted

urbanization are also threatening some species of butterflies to extinction by destruction or disturbance of their larval as well as adult food plants, feeding grounds, and shelters. The Travancore Evening Brown, the Malabar Tree Nymph, Bhutan Glory, and Kaiser-I-Hind butterfly are listed as endangered due to the wanton destruction of habitats in various parts of the subcontinent. Many of the most spectacular and endangered species have various levels of protection under CITES as well as under local legislation. However, there is a major trade in the spectacular tropical species for incorporation in ornaments and souvenirs. The international demand for insects is greater than most people realize. Next to bees and moths, butterflies are the most efficient pollinators of flowers to help turn them into food crops, fruits, and seeds so essential for the survival of man and animals. Wildlife farming, based on sustainable exploiting wild creatures, can help to save endangered species such as butterflies and their habitats.

Over the past decade, farmers in the Himalayan region have been complaining about decline in apple production and quality due to pollination-related problems. Apart from habitat alteration from highly diverse natural ecosystems far less diverse agrosystems, indiscriminate use of pesticides, and the overharvesting of honey through traditional honey-hunting methods have contributed to the extermination of both the diversity and abundance of pollinating insects. The general observation of farmers is that, in the past, there used to be a lot of insects such as wild bees, butterflies, and moths during the apple flowering season but now they have all disappeared. The scarcity of natural insect pollinators has, therefore, become a critical factor in inadequate pollination. The solution lies in supplementing populations of crop pollinators such as honeybees, bumblebees, stingless bees, solitary bees, etc. Farmers in Himachal Pradesh are using honeybee colonies of *A. mellifera* and *Apis cerana* for pollination. Hand pollination of apples is a common practice in Maoxian County of Sichuan, China. Awareness about the value of honeybees as crop pollinators has to be raised at all levels among planners, policy makers, beekeepers, or farmers. In western countries, farmers are already using honeybees and solitary bees (species of *Osmia*, *Megachille*, *Nomia*, *Xylocopa*, etc.) for pollination of different crops. The focus of beekeeping needs to change from conventional honey production to crop pollination.

16.5 Pollinators' Decline

Pollinators are products of millions of years of evolution and are eroding at a fast rate from the globe. The ecological consequence of contemporary agriculture can be viewed from various angles analyzing each component of agriculture—deforestation for expanding agriculture, soil, irrigation, fertilizer, pesticide, and agronomic practices with their influence on the environment of plants and thereby pollinators.

The perception of a worldwide pollinator decline appeared during the last decades and was motivated by various reasons: habitat impoverishment and declines of commercially raised pollinators (Richards 2001; Borges 2004). This pollinator decline was observed: in India, where pesticides cause severe insect mortality; natural

vegetation removing; and monocultures reducing floral diversity and thereby limiting the diversity of pollinators that could be supported (Borges 2004); in the Hindu Kush Himalayan region, where it appears that there is a reduction in productivity of cash crops, such as fruit and off-season vegetables, linked to a pollinator problem (Partap 2003). However, the perception is not consensual. The scientific community agrees on the fact that the expansion of agricultural area has greatly reduced the local biodiversity in tropical areas and degraded ecosystem services (such as nutrient cycling and water purification); however, there is no consensus about pollinator decline. Some researchers underline that there is a negative impact of habitat loss and agricultural intensification on pollinator diversity and, therefore, there is a decline of numerous pollinators: declines of solitary bees, bumblebees, and honeybees owing to habitat loss, agricultural intensification, and pesticide uses have been reported (Steffan-Dewenter et al. 2005); pollinators other than honeybees and bumblebees have many entries in the IUCN Red List (Steffan-Dewenter et al. 2005), which means that these species are endangered, vulnerable, or threatened (IUCN 2004). They also focus on the fact that the majority of important crops providing fruit or seeds benefit from animal pollination. Other researchers argue that only some pollinators have declined such as honeybees in America, some bumblebees and butterflies species. Moreover, they indicate that such declines are not of great importance because they will only cause an agricultural crisis for crops that are pollinator-dependent, pollinator-limited, and pollinator-specific, which is a minor segment. They also add that the loss of specialized pollinators mainly affects generalist plants that are buffered from such losses by the other pollinators that they also attract (Ghazoul 2005). But it is also true that in countries, such as Nepal and China, loss of pollinators does have an impact on agriculture—this can be seen with the apparition of hand pollination of apples in China, but also sociocultural effects such as cliff bees in the Hindu Kush Himalayan region of Nepal (Partap 2003).

Notwithstanding, observations of declines in pollinator populations have been observed in various part of the world. This is the case in America, where honeybee colonies dropped by 30 % between 1995 and 1996 (Allen-Wardell et al. 1998). Declines have also been observed in Europe and India, where, like in other countries that developed an important beekeeping activity with *A. mellifera*, honeybees are also suffering serious declines due to the parasitic mites *Varroa jacobsoni* and *Acarapis woodi* (Sihag 2001). It has also been found that overhunting of the megachiropteran fruit bats in the islands of the South Pacific reduced the fruit yields of some traditional harvests (Cox and Elmquist 2000). However, it should be noted that there is a tremendous diversity of pollinators, which are poorly known, and there seems to be some important spatiotemporal variations, which pleads in favor of a thorough study and over the duration of the pollinators and the populations trends (Borges 2004).

16.6 Declining Biodiversity

Decline in biodiversity results in decline in diversity of pollinators and vis-à-vis crop genetic diversity. About 75 % of the genetic diversity of agricultural crops has been lost since the beginning of twentieth century from the earth and 25 % of the world's

species present in the mid-1980 will be lost by 2015 (Raven 1988; WRI/IUCN/UNEP 1989; FAO 1993). More than 85 % of the 7,000 or so apple varieties grown in last century are now extinct in the United States (FAO 1993). In 1970, genetic uniformity of maize in United States caused almost \$ 1,000 million loss and yield reduced by as much as 50 %. In Europe, half of the breeds of domestic animals that existed in the beginning of the century have been extinct and one-third of the remaining in danger. Irish potato famine in 1840s is the result of genetic uniformity causing million people to die and million more to immigrate. Rice, one of the most important cereal crops in Southeast Asia, only ten varieties cover three-fourth of rice area, more than 30,000 grown before in the same areas in India. The cost of conserving biodiversity is far less than the penalty of allowing its degradation. Global extinction rate of species are accelerating at an alarming rate. Wilson (1988) estimated that 0.2–0.3 % of all species are lost every year. Range of 5–10 % of the tropical forest species may become extinct within the next 30 years (UNEP 1993). It is estimated that 60,000 species will be eliminated in the foreseeable future and 50,000 species will be at the risk of extinction in the next half of the century.

Currently, stocks of honeybees are experiencing many diseases and populations of wild pollinator species are declining in several regions (Kluser and Peduzzi 2007), raising concern that a potential global “pollination crisis” threatens our food supply (Withgott 1999; Kremen and Ricketts 2000; Richards 2001; Westerkamp and Gottsberger 1985; Steffan-Dewenter et al. 2005). In North America, the number of managed honeybee hives has declined almost 60 % since the mid-1940s, due to the increasing incidence of parasitic mites and other unidentified factors (National Research Council 2007; Oldroyd 2007; Stokstad 2007). Correspondingly, the diversity of wild bees has decreased greatly over much of Western Europe, mostly owing to habitat destruction (Biesmeijer et al. 2006; Fitzpatrick et al. 2007) despite the evidence that pollination shortages affect fruit and seed quality and quantity of many crops in many places (Klein et al. 2007). However, there is a strong evidence of pollinator limitation affecting global agricultural production (Aizen et al. 2008). However, it has been found that cultivation of pollinator-dependent crops has, on average, been expanding faster than that of nondependent crops in both developed and developing countries, so the demand for pollination service is rising at the same time that pollinator abundance and diversity are declining. In the near future, such opposing trends threaten crop yields, which could be averted either by further increases in inputs to compensate for a decline in productivity or by implementation of technical alternatives to traditional pollination practices. This bleak scenario calls for better information on the dependence of agriculture on pollinators: the estimates need to be more precise than the raw values reported so far (Klein et al. 2007).

16.7 The Decline of European Honeybees

Despite much of the world’s agriculture relying on pollination by European honeybees, their numbers worldwide have declined due to a range of natural and human-mediated causes. In the United States, Mexico, and Canada, both feral and managed honeybees declined by 25 % between 1990 and 1998 (Allen-Wardell et al.

1998; Loper 1995). In Europe, particularly France and Germany, the same species declined by about 10 % between 1992 and 2002. Honeybee “specialists consider all countries will become seriously affected” by this decline, which is expected to continue for at least the next few years (Dias et al. 1999).

16.8 The Decline of Asian Honeybees

Despite its economic usefulness, biodiversity of Asian hive bee *A. cerana* is suffering precipitous decline and is threatened with extinction in its entire native habitat. For example, in Japan, beekeeping with this native bee species has been completely replaced by European honeybee, *A. mellifera* and only a few beekeepers and research institutes are raising *A. cerana* colonies (Sakai 1992). In China, out of more than 8.5 million colonies of bees kept in modern hive, 70 % are exotic *A. mellifera* (Zhen-Ming et.al. 1992). Similarly, in South Korea, only 16 % beekeeping is with native *A. cerana* and remaining has been replaced by exotic *A. mellifera* (Choi 1984).

In Hindu Kush Himalayan range, beekeeping with *A. cerana* is being replaced by *A. mellifera* at such a fast rate that population of native *A. cerana* is declining to a level that is no longer viable. These countries include Afghanistan, Bhutan, Myanmar, Nepal, India, Bangladesh, and Pakistan (Verma 1994). A visit of some mountain areas of Northwest Frontier province of Pakistan in 1989 led by Eva Crane to conclude that *A. cerana* populations may soon become an endangered species (Crane 1992). Thus, the existing centuries-old and long-established craft of beekeeping with *A. cerana* has now almost got destroyed in its entire native habitat.

A. cerana remains till now a forgotten and completely ignored species. Therefore, from biodiversity conservation point of view, it will be disastrous to leave this important genetic resource at its own and definitely require research and development interventions for its conservation and sustainable uses both in natural and agricultural ecosystems.

16.8.1 Causes and Consequences of Declining *A. cerana* Diversity

In seeking ways to conserve genetic diversity of *A. cerana*, it is necessary to have a clear understanding of the major threats, which this bee species is facing in its own native habitat. Similar to any other threatened biological resources, decline in *A. cerana* population is also being threatened by human mismanagement, misguided scientific and economic policies, and faulty institutions. The major threats are described in the following sections.

16.8.1.1 Major Threat from *A. mellifera*

Many importations of *A. mellifera* in South and Southeast Asia have proved disastrous for beekeeping with *A. cerana*. When kept sympatrically, *A. cerana* and *A. mellifera*

colonies frequently robbed each other (Koeniger 1982). Another major problem is the transfer of parasites from one species to another. A parasite mite of brood and adults, *Varroa destructor* coexists with *A. cerana* and causes no serious damage to this native bee species. In several countries of Asia, where both these species are kept together, the parasite has infested *A. mellifera* colonies and became a serious pest to this unadapted host, now killing thousands of colonies every year. It is now well documented that through importations of *A. mellifera*, *A. cerana* populations in its native habitat are facing serious risk of extinction.

On the other hand, also native *A. cerana* populations are threatened by pests and parasites of exotic western honeybee *A. mellifera* for which *A. cerana* is lacking resistance. For example, there are several reports in the literature that Thai sacbrood virus disease, European foul brood, and possibly acarine disease jumped in to *A. cerana* and other Asian bee species from *A. mellifera* in Nepal, India, and other Asian countries killing large number of native bee colonies every year (Rana et al. 2003; Saville 2000; Allen et al. 1990). Akkratanakul (1990) reported three viral diseases affecting *A. cerana* namely, Thai sacbrood Virus, Kashmir bee virus, and *Apis iridescent* virus and all these viral diseases probably spread from *A. mellifera*.

Due to these afflictions, populations of *A. cerana* colonies practically reduced to the level of extinction, but through natural selections within two decades, normal population of this bee species stand restored from 10 % of surviving colonies (Reddy 1999; Ge et al. 2000; Ahmad and Partap 2000). These risk factors may vary between different habitat types, landscapes, and biogeographical region. The relative importance of these factors and in particular their combined effects on *A. cerana* genetic diversity loss are unknown.

Large-scale importations and multiplications of exotic *A. mellifera* in developing countries of South and Southeast Asia for better economic returns in terms of higher honey production has also become a myth. This bee species is now so seriously infested with parasitic mites, European foul brood, hornets/wasps/birds, wax moths that beekeeping with this exotic species require intensive treatment with chemicals to control these afflictions, which are very expensive and making this enterprise economically unviable. The intensity and the need for chemical treatment of *A. mellifera* colonies for mite, diseases, and pest control reveal that beekeepers in developing countries of South and Southeast Asia with large uneducated, ignorant populations in isolated areas are using chemical prescriptions indiscriminately and thus affecting the quality of honey (Verma 2008b).

16.8.1.2 Habitat Alteration

In the developing countries of South and Southeast Asia, habitat alteration (especially due to deforestation) from a highly diverse natural ecosystems to far less diverse (often monocultures) agroecosystems is adversely affecting native bee populations in the region. This is one of the most important threats often related to land-use changes on a regional scale that involve great reduction in the area of natural vegetation. Such habitat destruction could lead to loss of different types of flowering plants and bee

flora. Scarcity of bee flora due to environmental degradation not only leads to decline in colony numbers, but also creates “stress conditions” for living bee colonies and increases their vulnerability to the pests and diseases, hunting, and random population changes. Recent incidence of Thai sacbrood virus disease and European foul brood in *A. cerana* might have arisen due to the stress conditions created by environmental degradation.

Destruction of forest habitat for growing agricultural and horticultural crops adversely affects the availability of floral resources because many of the staple crops such as rice, wheat, barley, potato, etc. are of little or no value to honeybees. Due to increasing dearth of bee floral resources, colonies in the spring would not be able to build their own populations rapidly and this might force them to forego swarming or cause them cast smaller swarms that would reduce the probability of survival. In either case, the result would be an eventual decline in colony numbers.

Habitat destruction greatly limits the choice of honeybees to carefully choose a particular microhabitat in which to build nests and rear offspring and thus protect itself from the attacks of predators. In the absence of dense vegetation, nest sites are often visible from a long distance and colonies are not able to defend themselves effectively from the predators and they become more prone to absconding.

16.8.1.3 Pesticide Poisoning of Honeybees

Beekeeping and pesticides both have become essential inputs of modern agricultural management technology. By ignoring either of the two, global food production would be seriously impaired. Since the advent of synthetic pesticides several decades ago, the beekeeping industry, both in the developed and developing countries, has been incurring heavy losses. In developed countries, large-scale monoculture cultivation of crops and a high degree of mechanization had greatly amplified the problem of honeybee poisoning by pesticides. However, in recent years, education and public relations have achieved much in reducing bee losses due to pesticide poisoning in the developed world. In developing countries, all species of honeybee still face many risks, because agricultural practices often include insecticide application carried out in ignorance or indifference to the indispensability of bees. In all the developing countries of South and Southeast Asia, a large area of land is being brought under the cultivation of high-yielding exotic cultivars of crops and along with them their pests have also become introduced either through human error, accidentally, or lack of proper quantitative facilities. For the control of these pests, a large number of biocides are coming into use. Because of the lack of information, farmers in the region use blanket application without caring as to what and how much to use and when. Unlike developed countries, there is also lack of legislation to prohibit the use of pesticides to the extent that kill bees. Integrated pest management technologies for the protection of honeybees from harmful effects of broad-spectrum biocides are lacking. Such overreliance on chemical methods is adversely affecting environmental health including health hazards to human beings and decline in other nontarget animal populations. Among the latter, honeybees because of their social behavior run the highest risk of pesticide poisoning.

16.8.1.4 Diseases and Enemies

There are frequent reports of *A. cerana* colonies being affected by nosema, virus cluster, and sacbrood diseases in the Hindu Kush Himalayan region. Recently, the European foul brood disease has badly affected *A. cerana* colonies in Kathmandu valley of Nepal. Among the mites, *A. woodi*, *V. jacobsonii*, *Neocyphalaeps*, *Tropilaelaps* sp., and *Pymotes naferi* have been reported on *A. cerana*. Among these, acarine disease poses a serious problem (Verma 1987).

Among the predators, five different species of wasps pose a serious threat to beekeeping industry in this region. However, because of its shimmering and evasive behavior, *A. cerana* can resist the attacks of wasps better than *A. mellifera*. Two species of wax moths, *Galleria mellonella* and *Archoria grisella* are serious pests in *A. cerana* colonies as this native species of honeybee do not collect propolis to guard against the attack of moths. In recent years, Thai sacbrood virus disease has been reported from all countries where *A. cerana* is found. In early 1980s, the incidence and severity of this disease increased at such an alarming rate that more than 95 % of colonies infected in different countries were killed by this disease (Rana et al. 1986, 1987). This resulted in great economic loss to beekeeping with *A. cerana* in Asia not only in terms of honey and beeswax production, but also through adversely affected pollination services. The problem was particularly severe in the temperate region of the country where disease was more widespread than in tropical and subtropical region. The presence of Thai sacbrood virus in the diseased colonies of *A. cerana* in northern India was confirmed by conducting electron microscopic and serological studies (Rana et al. 1986, 1987). Different control measures recommended earlier to control the spread of sacbrood disease in *A. mellifera* were unsuccessful in *A. cerana* (Rana et al. 1986). However, about 5 % of colonies in the affected areas were resistant and escaped the attack of this disease. Detailed investigations on such colonies indicate that some mechanism of resistance to the sacbrood virus disease exists in *A. cerana*. In nature, this disease has a 5 years cycle, and after this period, about 5 % of the surviving colonies start multiplying in a normal way and normal population is then restored. Due to the incidence to Thai sacbrood virus disease in *A. cerana* in recent years, the beekeepers in the region had no choice but to adopt beekeeping with *A. mellifera*, which is not only free from this disease, but is also giving higher economic returns to the farmers. Consequently, *A. cerana* has been completely abandoned by the farmers in the region and it has now become endangered/threatened species of mere academic interest to the researchers in conservation biology.

16.8.1.5 Human Predations

Beekeeping in Asian region is marked by a long history of honey-hunting methods that killed most of the bees, destroyed the brood, and left no honey stores behind in the nest for consumption by bees during dearth periods. Such harmful exploitation by man resulted either in the loss of bee colonies or development of undesirable traits such as absconding or swarming colonies have the tendency to return to the same

nesting site each year, they are thus subjected to further harmful exploitation as these locations are well known to people. The net result of such human predation is both temporal and spatial decline in bee populations in its native habitat (Bishop 1992).

16.8.1.6 Population Vulnerability Analysis

According to Gilpin and Soulé (1986), loss of genetic diversity of species leading to its extinction is a systems phenomenon involving the interaction of processes and states. It is to be based on three interacting fields, i.e., population phenotype (PP), environment (E), and population structure and fitness (PSF).

When such model is applied in relation to loss of genetic diversity in *A. cerana*, the first field (PP) includes behavioral and genetic components such as frequent swarming and robbing, production of large number of laying workers, inbreeding depression, and drift inbreeding. A second field, the E is the context. It includes all abiotic and biotic factors that influence the population. In case of *A. cerana*, the loss of habitat quantity and quality as a result of rapid agricultural transformation and deforestation in the region and pesticide hazards due to their indiscriminate use are important abiotic components. The biotic components include introduction of exotic *A. mellifera*, epidemic of sacbrood virus disease, and human predations as a result of traditional honey-hunting methods. PP and E together determine the third field, the PSF. This is the field in which dynamic consequences of interactions of PP and E are manifested in terms of patch distribution, population fragmentation, demographic randomness, reduced effective population size, growth rate, and distribution leading to stochastic and deterministic extinction. The decay in one component can exacerbate not only itself, but also the behavior of other components.

16.9 The Decline of Other Bee Species

In natural systems, particularly biodiversity hotspots such as tropical rainforests, the decline in pollinator numbers has a more significant effect, because their services are essential to maintain that diversity. The more plant species are present in a habitat, the less is the access for each species to the pool of pollinators. As each pollinator declines and the “pollination limitation” increases, the risk of extinction for any plant species also increases (Vamosi et al. 2006). Pollination limitation, involving reproductive shortfall or failure of seed set, is thought to be in the range of 50–60 % in rare plant species or plants found in fragmented habitats (Allen-Wardell et al. 1998), and some research suggests more than 60 % of plant species studied are pollination-limited (Burd 1994). Forests have the added burden of habitat loss due to agricultural encroachment, habitat fragmentation, and the invasion of Africanized bees (Roubik 2000).

Aside from biodiversity hotspots, there are a number of other natural ecosystems particularly susceptible to the effects of pollinator decline. In tropical communities dominated by large tree species, such as figs, where each fig species is dependent on one or two species of fig wasps for pollination, and where 80 % of the vertebrate

species rely on the fruit as the basis of their diet, loss of a few pollinator species can be catastrophic to the entire ecosystem. This is also the case on islands, where pollinator guilds are often depauperate even without human interference, and a number of plant species may rely on a single pollinator (Allen-Wardell et al. 1998).

One particularly important area for pollinators is the interface between agricultural lands and natural ecosystems. When managed pollinators such as European honeybees are not capable of pollinating a crop to full capacity, other bee species from surrounding areas may be able to complete the task (Kremen and Ricketts 2000). However, the impact of pesticides and other human-generated activities may extend for some distance into natural ecosystems, affecting both the crops and native plant species.

16.10 Reasons for Pollinator Decline

Pollinator decline has been a global issue for many decades as natural ecosystems were cleared to make way for agricultural systems, particularly monocultures. This decline has accelerated dramatically in recent years because, in addition, a number of factors such as climate change, the spread of bee parasites and diseases, the overuse of pesticides, the spread of Africanized bees and other invasive species, and the introduction of genetically modified organisms (GMOs) appear to have compounded the situation.

16.10.1 *Parasites and Diseases on European Honeybees*

Global populations of European honeybees have suffered for many years from a range of diseases such as European and American foul broods, with parasites causing additional problems in recent years. The honeybee tracheal mite (*A. woodi*) was discovered in the 1920s and slowly spread throughout the world, reaching the United States in the 1980s. Today, it is found in all countries except Australia, New Zealand, Scandinavia, and Canada. The mites infect the tracheal walls of young adult bees and shorten their lives, reducing honey production and pollination efficacy (Morse 1978). The *Varroa* mite (*V. jacobsoni*), a more serious pest of honeybees, is cosmopolitan throughout the world except a few isolated countries such as Australia. In 1987, it was detected in Florida and within a short period had spread across most of the United States. The mite feeds externally on bee larvae, pupae, and adults and, if left unchecked, will kill most bee colonies in 7 months to 3 years (Ritter 1981).

16.10.2 *Habitat Changes*

One of the major causes of native pollinator decline around the world is changes to habitat. This may include habitat loss and reduction, particularly in areas where

natural ecosystems are replaced with agricultural systems; habitat fragmentation, where natural ecosystems survive but in patches too small to support sustainable pollinator populations; and habitat disturbances, where human activities disrupt pollination systems even when the habitat itself remains intact (Kremen and Ricketts 2000). An additional complication is the replacement of natural habitat with monocultures. Even for bee species that will feed on the crop and effectively pollinate it, the bees may be unable to find suitable nesting sites or alternative flowers when the monoculture is not in flower (Dias et al. 1999).

Natural forests that play a vital role in maintaining ecological balance, providing energy, animal fodder and timber, and recharging water tables, are being degraded day by day causing habitat loss of other life system and ultimately threatening biodiversity and associated pollinators in Nepal. A constant rise in the population, higher rate of deforestation, and overexploitation of resources with expansion of farm lands for agriculture and rearing livestock, cause a continuous depletion of the forest resources. The lowland and midhill fauna are more endangered than mountain fauna. Kaiser-I-Hind is the rare species of butterfly listed in IUCN Red Book. This is mainly due to greater human activity in lowland and midhills. Since 1945, 17 % of the earth's vegetative land (1.2 billion/ha) degraded to an area equivalent to China and India together. To meet the need, the agriculture is expanding and pollinators' habitat is being lost so rapidly that sustainable agriculture is in jeopardy.

16.10.3 Threats to Pollination Systems

The collapse of pollinator mutualisms has been identified as one potential consequence of anthropogenic land-use change (Kearns and Inouye 1997; Allen-Wardell et al. 1998; Kearns et al. 1998; Wilcock and Neiland 2002). Declines in pollinators have been reported from most continents (Kearns et al. 1998; Kevan and Phillips 2001). Land clearance, fragmentation, agricultural practices, herbicides, pesticides, and the introduction of exotic plant and pollinator species (Table 16.1) have all been implicated in a serious decline in pollinators that has been referred to as a "pollination crisis" (Buchman and Nabhan 1996). Loss of or interruption to pollinator services may have several outcomes. The most obvious result is a loss or reduction in seed set, however, impacts may also extend to reduced offspring vigor as a result of self-pollination, decreasing heterozygosity, and in the increased expression of deleterious traits, resulting from inbreeding (Kearns and Inouye 1997). Ultimately, loss of seeds, fruits, or plants will affect animals that rely on these resources.

Similar to most tropical landscapes, the Wet Tropics have been subjected to processes of fragmentation over the last 100 years or so. Plant species "marooned" in these fragments may or may not be part of viable populations—and it may take much longer than 100 years before this becomes evident. Pollination and the subsequent reproductive performance of plants in fragments becomes a crucial issue. Understanding the changes that will occur to pollination processes and outcomes in fragments is an essential first step in managing these changes and attempting to

Table 16.1 Summary of threats to pollination systems

Threat	Effect	Impacts
Fragmentation	Reduced population size	Increased genetic drift, in-breeding depression, increased threat of extinction, reduced pollen dispersal, and reduced fitness (Rathcke and Jules 1993; Kearns et al. 1998)
	Isolation	Increased reproductive success (Cunningham 2000)
	Hostile matrix	Temporary reduction in pollinator activity (Becker et al. 1991)
	Alteration of visitor behavior	Genetic erosion of small populations (Cane and Tepedino 2001; Ghazoul et al. 1998, Oostermeijer et al. 1998)
		No reduction of reproductive success, substantial between- and within-site variability (Costin et al. 2001)
		Effect of isolation tied to pollinator mobility (Law 2001)
		High genetic differentiation among geographically close patches (Dutech et al. 2002)
		Pollen clogging by generalist pollinators (Kunin 1997; Groom 2001)
Agricultural practices	Land clearing	Pesticides reduce pollinator numbers (Batra 1981)
	Pesticide spraying	Poisoning of pollinators resulting in death, behavioral changes, and reduced mobility (Johansen 1977)
	Herbicide spraying	Contamination of pollen and honey (Kearns et al. 1998)
	Extensive monocultures	Herbicides reduce availability of nectar plants, remove nesting sites, and destroy larval food sources for pollinators
	Grazing	(Kevan 1975a; Kearns et al. 1998; Richards 2001)
	Resource depletion	Grazing changes nesting sites, decreasing water availability, and replacement of native grass species with introduced pasture grasses (Kearns and Inouye 1997)
Invasive species	Displacement of pollinators by feral competitors	Feral honeybees compete for pollen normally available to native pollinators, altering pollen dispersal patterns through foraging that differs from native pollinators, and depleting nectar supplies to nectar-feeding pollinators (England et al. 2001)
	Displacement of native plants	Introduced bees implicated in successful spread of exotic plant species where native animal species are not suitable pollinators (Stout et al. 2002)

ensure the long-term future of our forests. That having been said, there are almost no data available on this topic. The study by Law and Lean (1999) on *Sygygium cormiflorum* did demonstrate that visits by vertebrates to the flowers were skewed in favor of bats over birds in fragmented situations.

16.10.4 Pesticides

In many parts of the world, pesticides are used to control insect pests on a large scale, but pollinators (as well as the natural predators of the pests) are usually more susceptible to the pesticide than the target insects. Widespread use of pesticides in many parts of the world has reduced the overall numbers of pollinators (Pimental et al.

Table 16.2 Insect flower visitors in sprayed and nonsprayed mustard crop in Chitwan. (Source: Thapa 2006)

Common name	Scientific name	Sprayed field	Nonsprayed field
Lady beetle	<i>Coccinella</i> spp.	—	+
Bumble bee	<i>Bombus</i> spp.	—	+
Yellow banded wasp	<i>Xylocopa</i> spp.	—	+
Rice skipper	<i>Pelopidas methias</i>	—	+
Tiger moth	<i>Nyctemas lactinia</i>	—	+
Cowpea pod borer	<i>Lampides boeticus</i>	—	+
Cynthomid fly	<i>Cyntomis passalis</i>	—	+
Mustard sawfly	<i>Athalia proxima</i>	—	+
Nymphalid butterfly	<i>Presis atlites</i>	—	+
Mud wasp	<i>Chlorion labatum</i>	—	+
Short horned grasshopper	<i>Oxya</i> spp.	—	+
Green stink bug	<i>Nezara viridula</i>	—	+
Blister beetle	<i>Mylabris</i> spp.	—	+
European honeybee	<i>A. mellifera</i>	+	+
Asiatic honeybee	<i>A. cerana</i>	+	+
Rock bee	<i>Apis dorsata</i>	+	+
Syrphid fly	<i>Milesia</i> spp.	+	+
Tabanid fly	<i>Tabanus</i> spp.	+	+
Hymenopteran wasp	<i>Sphex macuta</i>	—	—

+ present, — absent

(1992)) and this, particularly in the case of rare insect pollinators and/or rare plants, can have a devastating impact on pollination systems (Nabhan and Buchmann 1997). There is also a concern that sublethal doses of pesticides may disrupt the pollinating behavior of all types of bees and render them more susceptible to diseases and parasites (Allen-Wardell et al. 1998).

16.10.5 Case Study

Widespread usage of pesticides is a major threat to pollinators worldwide, especially with the onset of modern large-scale agricultural practices. This results in the requirement of large number of commercial bee colonies for pollination. These pollinators feed on the contaminated flowers, which has resulted in bee poisoning becoming the most important problem for beekeepers throughout the world. Honeybees are susceptible to almost all pesticides used commercially to control pests and diseases. Poisoned bees not only die but, even on exposure to sublethal doses, suffer disruption in dance behavior and thereby breakdown of accurate communication of information about resources. Poisoned queens are unable to maintain control over the hive and are often superseded. A survey conducted very recently to record flower visitors in insecticide sprayed and nonsprayed mustard crop is presented in Table 16.2. The insect flower visitors in nonsprayed field were recorded more than three times higher

(19 insect species) than those in sprayed field (6 species). It is clear that pesticide spray has been one of the various factors for pollinator decline.

16.10.6 Climate Change, Pollinator Declines, and Competition

Anthropogenic changes in climate are strengthening and are likely to influence the occurrence and magnitude of competition for pollination by altering ecological context. These influences are virtually certain to be difficult to predict. Climate change should directly and indirectly affect the abundance, geographic range, vigor, phenology, and behavior of both plants and pollinators, all of which can influence interactions among plant species mediated through shared pollinators (Hegland et al. 2009).

Climate change may most immediately affect plants by altering their resource status—directly through increases in availability of carbon (via increased atmospheric CO₂), or indirectly by increases in nutrients such as nitrogen and phosphorus (mobilized via, for example, increased warm-season precipitation). No work has yet directly investigated how climate change might affect competition for pollination and other effects on flowering communities are just beginning to be explored (Price and Waser 1998, 2000). Immediate plastic responses of plant traits related to pollination are known in some cases, but vary across species. Examples include increased, decreased, or unchanged rates of nectar production (Osborne et al. 1991; Rusterholz and Erhardt 1998), increases in numbers of flowers produced by some but not all species (Osborne et al. 1991), and other changes that might differentially alter attractiveness to pollinators (Wookey et al. 1993). How such responses would affect any element of competition for pollination in a wild community (and with independent responses of different species of plants and pollinators to climate change) requires additional investigation.

Declines in pollinator populations have been reported around the globe (e.g., Buchmann and Nabhan 1996; Biesmeijer et al. 2006; Colla and Packer 2008; Goulson et al. 2008) and may in part be the result of climate change (Allen-Wardell 1998; Committee on the Status of Pollinators in North America 2007). In turn, declines in plant populations may be linked to those of pollinators (Biesmeijer et al. 2006). With such interdependent population dynamics, forecasting the outcome is uncertain—the future may bring a shortage of pollinators relative to plants at some times and places, and a shortage of plants relative to pollinators in others. When there is a shortage of pollinators, forms of competition for pollination derived from changes in visit number should increase (Vamosi et al. 2006). In this situation, pollinators would face a world of relatively undervisited flowers (consequently rich in nectar and pollen), and might therefore reduce their visitation to less rewarding species or avoid these entirely. Either option would reduce success through both female and male sex functions for the undervisited species. Conversely, if there is a shortage of flowers relative to pollinators, pollinators might broaden their diets, and therefore increase competition through mechanisms derived from a change in visit quality. Pollinators facing a world depleted of flowers should be less choosy and deposit more foreign pollen. Over the longer term, as declines in populations of plants and

animals lead to local extinctions (Biesmeijer et al. 2006; Colla and Packer 2008; Goulson et al. 2008), and as novel communities of mutualists and antagonists are assembled (Pitelka et al. 1997; Memmott et al. 2004), competition for pollination is likely to change in unpredictable ways, but could well intensify. Finally, it seems almost certain that climate change will affect the ecological context by altering the phenological synchrony of interacting species. After all, different species of plants and pollinators respond to different environmental cues in individually differing ways (Inouye et al. 2000; Lyon et al. 2008). Changes in phenology will alter not only the extent to which different plant species overlap in flowering time, but also their synchrony with different pollinator species (Memmott et al. 2007). Experimental studies of such effects are exceptionally challenging because of the difficulty of manipulating plants on a scale that will also affect mobile pollinators.

Today, alarming declines in the health and populations of pollinators pose a significant threat to the integrity of biodiversity, to global food webs, and to human health and survival. Habitat loss, fragmentation and degradation, pesticides, invasive species, parasites and diseases, exploitation and climatic change, etc. are major causes for pollinator decline (William and Osborne 2009). Disruption of pollinator systems and declines of certain types of pollinators has been reported in every continent except Antarctica (Kearns et al. 1998).

An estimated 62 % of all flowering plants may be suffering reduced regeneration from seeds as a result of pollinator scarcity (Burd 1994). Over the past decade, farmers in Himalayan region have been complaining about decline in apple production and quality due to pollination-related problems. Apart from habitat alteration from highly diverse natural ecosystem for less diverse agrosystem, indiscriminate use of pesticides, and the overharvesting of honey through traditional honey-hunting methods have contributed to the examination of both the diversity and abundance of pollinating insects. The number of commercially managed honeybee colonies in United States has declined from 5.9 million in the 1940s to 4.3 million in 1985 and 2.7 million in 1995 (Maheshwari 2003).

16.10.7 Invasive Species

Invasive plant species, often not requiring pollination to reproduce, are able to move in and displace native plant species, disrupting the ecology of both local plants and their pollinators. This is particularly destructive on small islands. Invasive animal species may impact the pollinators through competition with or predation on local pollinators (Kremen and Ricketts 2000).

16.10.8 Other Factors

Two other factors whose impact on pollinators is not so clear are Africanized bees and the role of genetically engineered crops. Long-term studies in South America have shown that the invasion of the aggressive and adaptable Africanized bees into

native ecosystems has undoubtedly caused the loss of some native species of bees, but their impact on overall pollination systems is still under review (Roubik 2000), given that they have negative effects in some regions, but neutral and occasionally positive effects in others. Genetic engineering and “the rapid development of transgenic crops raises additional causes for concern among specialists on bees” (Dias et al. 1999). The practice of incorporating the insecticidal Bt gene into crops has raised concerns about the effect of pollen from these plants on pollinators, but so far the evidence is scant. Some studies have suggested GM pollen from a number of crops reduces the survival rate of caterpillars such as the Monarch or Wanderer butterfly (*Danaus plexippus*), as well as European honeybees (Conner et al. 2003).

16.10.9 Honey Hunting

Thapa (2006) reported that as consequence of honey-hunting methods *Apis laboriosa* colonies were rarely observed in Chitwan, Nepal. Similarly, as a result of habitat destruction and honey hunting, the wild honeybees, *Apis dorsata* has been declining in Chitwan by more than 50 % in the year 2004 as compared with 2003 (Pokhrel 2006).

16.10.10 Introduction of New (Exotic) Species

A. mellifera Lin. is the exotic honeybee of European origin and imported in the country for honey production. Partap et al. (2000) in the field study in Kathmandu valley reported that worker bees of *A. mellifera* carried significantly heavier pollen loads from both peach and plum flowers than those of *A. cerana* worker bees. However, studies conducted on the pollination of strawberry showed that *A. cerana* collected heavier pollen loads during morning and noon hours showing time and crop specificity. But the introduced species, *A. mellifera* completely replaced domesticated native *A. cerana* bees as indicated by the absence of worker bees during early, mid, and late hours under Chitwan condition. In addition, beekeepers in terai regions are keeping *A. mellifera* and slowly replacing the native honeybee *A. cerana*, and thus a decline of *A. cerana* bees has been recorded in the terai regions.

With the development of a beekeeping industry, honeybees, particularly *A. mellifera*, were introduced into many areas of Asia for such bee products as honey, pollen, royal jelly, and propolis, etc. However, as the business benefits from the introduction of *A. mellifera* colonies grew, many problems emerged. As mentioned above, these included foraging competition, mating interference, robbing, and the transmission of diseases, etc. The introduction of *A. mellifera* colonies has also had an enormous impact on the native honeybee species in some areas of Asia (Japan: Sakagami 1959; India: Atwal and Sharma 1971; China: Ji et al. 2003; Yu and Han 2003; Yang 2005; Nepal: Partap 1998).

A. mellifera was first introduced in China in the 1920s (Kuang and Kuang 2002). On introduction, this western honeybee not only proved adaptable to a new environment and produced higher yields of bee products, but also royal jelly and propolis, which cannot be collected from *A. cerana* colonies because of their extremely low productivity. Since then, this productive species of honeybee began to be widely adopted in Chinese beekeeping.

While enjoying the high profits of these bees, the negative aspects have been widely neglected and few, if any, had realized the strong impact of *A. mellifera* on the environment and the local honeybees, especially *A. cerana*, until the 1980s. An investigation was launched and conducted by the *A. cerana* Association of China. The results showed that *A. cerana* has become extinct in the Daxing-anling forest areas in the northeast and in Xin Jiang province in the northwest. In the Northeast Plain and North-China Plain areas, all the *A. cerana* bees in man-made hives have absconded (Yang et al. 1982). In the whole northeast zone, only in the Changbai mountain areas *A. cerana* bees can be found in wild and man-made hives. The plain of drainage area of the Yangtze River where millions of *A. cerana* colonies were kept in the past are now hard to find. In the southern provinces, such as Jiangxi, Hunan, Fujian, Guangdong, Guangxi, and Hainan, there are still many *A. cerana* colonies, but their distribution area has shrunk greatly. Compared with those areas above, *A. cerana* colonies in the southwest are in a better condition, particularly in mountainous areas where many *A. cerana* bees can be found living in tree holes, caves, and man-made hives in Yunnan province and Tibet (Yang et al. 1982).

In conclusion, the introduction of *A. mellifera* caused great losses of *A. cerana* colonies. The population of *A. cerana* colonies is now estimated at not more than 1 million, a decrease of some 60 % compared with the number before the introduction of *A. mellifera* and their distribution has shrunk by 75 % (Yang 2005).

In the case of the introduction of *A. mellifera* in Asia, as early as 1959, Sakagami had noticed the impact of *A. mellifera* on *A. cerana* in Japan. In Nepal, Partap (1998) reported that plants and fruits were in shortage of pollination because of the population decrease of *A. cerana* bees, which was caused by the introduction of *A. mellifera*. Also, even in Europe, with the rapid development of beekeeping at the beginning of twentieth century, many beekeepers preferred to raise some subspecies such as *A. mellifera ligustica* and introduced them from other areas, which caused the local extinction of native subspecies (Ruttner 1988).

Moritz et al. 2005 recognized the severe disaster caused by the introduction of *A. mellifera* to tropical ecological systems and pointed out that local honeybees or other pollinators suffered from the introduced species through food competition or diseases. This resulted in a reduction of biodiversity and an imbalance of the whole ecological system.

During the mating season, both the virgin queens of *A. cerana* and *A. mellifera* can attract heterospecific drones (Yang 2001; Ji et al. 2003; Wang et al. 2003). However, the *A. mellifera* drones, which are much stronger fliers than *A. cerana* drones, can trap the *A. cerana* queens, although they cannot always mate with them successfully because of the differences in copulatory organs. Their encirclement behavior can inhibit successful mating between *A. cerana* queens and drones. In some areas with

many *A. mellifera* colonies, most of the virgin *A. cerana* queens were trapped by *A. mellifera* drones, and only 16 % of *A. cerana* queens were able to mate successfully. More than 80 % *A. mellifera* queens could successfully mate with conspecific drones, although there was interference by *A. cerana* drones (Wang et al. 2003). This resulted in the population decline of *A. cerana* bees in some areas in recent years. In some areas, they are threatened because their declining population is insufficient to support the community and honeybees are dying out. The decrease or extinction of the native honeybees is a definite threat to the balance of ecology and some plant species could also become extinct because of insufficient pollination (Yang 2005).

In Southeast Asia, exotic species of plants and animals have been introduced in different countries with more or less success. In Thailand and Vietnam, the exotic honeybee *A. mellifera* has been introduced more than 20 years ago, allowing the development of a strong beekeeping industry, but provoking a rapid decline of the number of apiaries populated with the native species *A. cerana* (Pechhacker 2000). In Lao PDR, where *A. mellifera* is not present, there have been various attempts to introduce it in order to develop the beekeeping industry, but with no positive result up to now. However, as has been highlighted in numerous surveys, the introduction of exotic species is a risky solution. A 21 article published in the *Lao Journal of Agriculture and Forestry* highlights four kinds of risks linked to the introduction of *A. mellifera* in Lao PDR (Sengngam et al. 2006):

1. *Modification to the balance of the species*: This would be related to the disorders of the mating between males and queens of the same species.
2. *Food competition*: The introduced bees (*A. mellifera*) exploit the same type of flowers as *A. cerana*.
3. *Genetic-related risks*: Any introduction of genetic material can have unexpected effects, sometimes undesirable (e.g., Africanized Honeybee in South and Central America).
4. *Pathological risks* due to the possible dissemination of diseases and parasites of *A. mellifera* onto *A. cerana*.

As indicated by Carlos Vergara (2005), “in most cases, the introduction of exotic species has been performed without prior assessment of possible negative impacts of the pollinators on native ecosystems.”

Apart from the possible undesirable effects of exotic bee species regarding the competition with native flower visitors for floral resources (Kato et al. 2008) and the spread of parasites or pathogens (Anderson 1995; Fang 1995), it is also stated that there is a risk of increased weed problems due to a possible pollination of exotic species by introduced exotic bees, and so a progressive change in seed set of native plants (Goulson 2005).

Most authors concur that honeybees are not particularly aggressive to other insects while foraging, so that impacts on other species occur primarily through exploitative competition (Schaffer et al. 1979, 1983; Thorp 1987; Roubik 1991). However, honeybees have been found to displace smaller species from flowers by physical disturbance (Gross and Mackay 1998). Honeybees do attack nests of other honey-storing species to steal the honey, a behavior that may have contributed to the decline of

A. cerana in Japan (Sakagami 1959). When visitation rates are measured, not only exploitative competition can occur, but also interference competition, with honeybees displacing native bees from floral resources by their physical presence. For example, in Japan, Sakagami (1959) found that at an artificial nectar source, *A. mellifera* and *A. cerana* attacked each other and eventually *A. cerana* were excluded.

16.11 Regions Where *A. mellifera* Does Not Naturally Occur, but Other Species of *Apis* Are Endemic

Introductions of European *A. mellifera* into Asia for honey production have had mixed success. Recently, beekeeping in China has been concentrating on *A. m. mellifera*, causing a considerable decline in *A. cerana* beekeeping (Li 1998). The problem could potentially be grave, because drones and queens of both species readily mate but do not produce viable offspring (Ruttner and Maul 1983). *A. cerana* queens may thus have poor mating success if many *A. mellifera* drones are present. Indeed, Li (1998) showed that *A. cerana* queen mating success was influenced by *A. mellifera* drones, sometimes causing a complete lack of successful mating of *A. cerana* queens. Competition for floral resources with other bees in general and nest site competition between *A. mellifera* and the other cavity-dwelling *Apis* species in Asia (*A. cerana*, *A. koschevnikovi*, *A. nigrocincta*, and *A. nuluensis*) in particular may also occur (for a recent review on the relatively few documented cases of the interactions between introduced *A. mellifera* and wild bees in Asia, see Paini 2004). Whether this competition results in true invasions *sensu stricto* Lonsdale (2004) may be questioned because introduced *A. mellifera* also suffer from competition with native species. In regions where other honeybee species naturally occur, *A. mellifera* colonies are often outcompeted. For example, the native honeybee species *A. cerana* and *A. dorsata* negatively affected the growth of European *A. mellifera* colonies in a forest ecosystem through aggression and robbing of stores (Manila-Fajardo and Cleofas 2003). In spite of the diversity in the forest ecosystem, *A. mellifera* failed to exploit the nectar and pollen sources of most plant species (Manila-Fajardo and Cleofas 2003). Moreover, competition for nectar between *A. mellifera* and *A. cerana* has been shown in feeding experiments (Sakagami 1959). Introduced European *A. mellifera* often suffer from novel parasites and diseases. For example, the devastating global spread of the parasitic mite *V. destructor* (Anderson and Trueman 2000; Oldroyd 1999) resulted from *A. mellifera* introductions into Asia and a host shift from *A. cerana* to the western honeybee. In this light, it may not be surprising that there are no reports yet of large feral populations of *A. mellifera* in Asia despite repeated introductions. This suggests that the introduction of European subspecies of *A. mellifera* have not caused invasions with large effects on biodiversity in Asia so far. But, this does not exclude the possibility that introductions of African subspecies, which are better adapted to tropical conditions, may result in quite a different scenario.

16.12 Diseases and Pests

Nepal did not know about any serious bee disease until 1980, when the serious outbreak of the sacbrood disease caused by the Thai sacbrood virus, occurred first along the eastern border areas. The disease spread so fast that within 4 years it covered the entire length of the country, and reached to peak in western border areas within 3 years. During the time, almost 90 % of the colonies lost (Kafle 1983; Shrestha and Shrestha 2000). By 1984, the disease started to subside and the bees started to regain normal condition again from the eastern border. The Asian mite, *V. jacobsoni* is associated with *A. cerana* and *A. dorsata* bees, but causes no serious problem to them, but it is fatal to *A. mellifera* colonies. *A. mellifera* colonies may collapse in the near future unless timely precautionary measures taken. Farmers' training to beekeeping in modern hives, regular supervision, and seasonal management seem necessary to establish good apiaries free of disease and pests.

16.13 Pesticide Pollution

Insects are viewed from the harmful perspectives and aimed at killing them through several means including indiscriminate use of deadly chemicals. The demand of pesticides increased in Nepal with the introduction of high-yielding varieties of crops, massive input of chemical fertilizers, and irrigation facilities, which not only improved the agricultural productivity considerably, but also created multifaced problems resulting in a large amount of crop losses and turning ecological sound farming into pest problems, crop loss, and pesticide pollution (Thapa 1994; Thapa et al. 1995). The use of chemical pesticides is concentrated in standing crops and postharvest loss protection in agriculture, and control of malaria and black fever in public health sectors. Several studies estimate that the use of pesticides in agriculture worth of more than Rs. 50 million and this guess could be as high as Rs. 500 million in agriculture, agroforestry, and human health. Among the crops, cotton receives the highest amount of pesticides (2,560 g/ha) followed by tea (2,100 g/ha) and then vegetable farming (1,450 g/ha) as compared with the national average of 142 g/ha.

Regarding the type and volume of pesticide used by the farmers, both the types and volumes decline from terai to mountain sharply (Thapa et al. 1995, 2003). Commercial farmers have no sufficient understanding of pesticide handling, safe application, and pollution to the environment.

Both the misuse and excessive use of pesticides disturb the natural ecosystem and produce serious environmental problems adding costs in four ways to the people: (1) health-related expenses, (2) environmental pollution, (3) yield loss due to nontarget pesticide application resulting in pesticide-induced pest resurgence and destruction of natural enemies, and (4) financial burden both to poor farmers and the country as a whole (Baker and Gyawali 1994). Pesticide problem on pollinators is severe in the developed country such as United States (loss of about US\$ 320 million per year) and is equally important for other countries as well. After the heavy use of chemical

pesticides, all domesticated bees were wiped out in Ilam and Nuwakot and many colonies were destroyed in Chitwan (Sharma 1994; Thapa 1994, 1999).

A survey conducted very recently to record flower visitors in insecticide-sprayed and nonsprayed mustard crop is presented in Table 16.2. The insect flower visitors in nonsprayed mustard field were recorded more than three times higher (19 insects species) than those in sprayed field (6 insects species only). It is clear that pesticide spray has been one of the various factors for pollinators' decline. Therefore, it is essential to survey and collect insect species in various crop plants during their flowering periods, identify and conserve them, and explore their potentiality as crop pollinators.

16.14 Global Warming and Climate Change

Global warming is caused by something known as the green house effect, brought about by the ability of the atmosphere to be selective in its response to different types of radiation. The CO₂ (56 %), CH₄ (14 %), CFCs (23 %), and N₂O (7 %) are the main green house gases, of which CO₂ accounts more than 50 % for global warming. Atmospheric temperature will increase by 1.5–5.50 °C by the year 2030, causing loss of 10–15 % arable productive coastal land due to the melting of polar ice caps and raising of sea level, and CO₂ concentration increased from 290 ppm 100 year ago to 350 ppm today and is likely to go up to 440–500 ppm by 2100. European scientists have warned that a long-term two degree Celsius or more increase in the average global temperature could threaten Latin America's water supplies, reduced food yield in Asia, and rise in extreme weather condition in the Caribbean. Global warming alters precarious habitats or eliminates food supplies. Based on the sample of 1,103 land plants and animals, it has been estimated that 15–37 % would eventually become extinct as a result of climate change expected by 2050. Similarly, Californian scientists analyzing 9,787 living and 129 extinct bird species, reported that one-tenth of all bird species could be extinct by 2,100 and by then another 15 % could be on the brink of extinction. The vulture population of India has crashed down by 95 % in the last decade. These birds keep down insect populations, spread seeds, and pollinate flowering plants and scavenge on carrions. More intense and erratic rainfall events are expected to be a feature of climatic change. The fate of agriculture of Nepal lies on rainfall, early rainfall in April, May in the hills and mountains to sow the seed of corn with some rainfall in June and heavy rainfall in July for rainy season crops. If this does not happen, then entire hills will become famine-stricken area. Similarly, agriculture in terai area starts with early rainfall in July and heavy but discontinuous rainfall up to the end of September (some time up to December). First rain helps paddy plantation and second rain helps wheat plantation. If one failed, the second crop is also likely to be failure unless artificial pumping ground water or irrigation water is provided. Climatic changes have been realized for past few years. Hills are getting more landslides and glacial lakes outburst and more of cloud burst brought in scattered rain. Terai is experiencing heavy rain in some areas and drought condition

in other areas. It appears that country's modulation capacity to absorb heavy flood and increase low flow has now changed because of environment degradation, which directly influences habitat and its biotic flora and fauna.

16.15 Impacts of Pollinator Declines

Since agricultural activities were first recorded, there have been shortages of pollinators. Today, it seems that pollination systems in many areas of agriculture are threatened by the inadequacy or lack of sustainable managed, indigenous, or imported pollinators. Pollinator shortages can adversely affect crop production and commodity markets. It is widely believed that pollination is in such serious jeopardy from the viewpoints of agricultural productivity and food security (Kevan et al. 2001) that the Convention on Biological Diversity and the Food and Agricultural Organization of the United Nations have recently (1998–2000) taken on leading roles internationally in this area.

16.16 Do Pollination Deficits Exist in Agroecosystems?

The oldest recorded examples of pollination and pollination deficit in crops are for sycomore (also known as sycamore) figs, *Ficus sycomorus* (Amos ca. 760 BC) and for date palm, *Phoenix dactylifera*, and Smyrna figs, *Ficus carica* (Herodotus 485–425 BC).

Although the sycomore fig is native to central eastern Africa and Yemen, it is also widely cultivated in Egypt and Mediterranean countries, where its pollinators (*Ceratosolen arabicus*: Hymenoptera: Agaonidae) are absent (Galil and Eisikowitch 1968, 1969a, b, 1974). Theophrastus (372–287 BC) recorded the lack of seeds in Egyptian figs and Galil (1967) noted that there were no wasps associated with figs from ancient tombs. How the plant spread beyond the reaches of its pollinators is unknown, but its range included Egypt by at least 3000 BC (Galil 1967). For the unfertilized fruit to develop, it must be scraped in the manner described by Theophrastus (372–287 BC) and Galil (1967), often with a special knife (Henslow 1892, 1902; Keimer 1928). Depending on the translation, Amos (760 BC) describes himself variously as a fig scraper, piercer, dresser, or gatherer. Nevertheless, whatever his occupational designation, he clearly understood how to produce sycomore figs without pollinators.

The date palm is dioecious and appears to be pollinated by wind and bees (Free 1993; Roubik 1995). Because male palms are not fruitful in the sense of agricultural production, only female palms have been retained. The result, even about 3,000 years ago in Mesopotamia, was that hand pollination using male inflorescences taken to the female trees was necessary (Tylor 1889; Meeuse 1981). Herodotus (485–425 BC) also described this practice; however, he was under the impression

that it also involved a gall fly, and he mixed the techniques used for the anthropogenic pollination of dates and *F. carica*. Pollination was probably associated with festivals of spring and fertility in the region at the time of the Prophet Mohammed, who reportedly discouraged such festivals and only reluctantly recognized the need to hand pollinate dates (Margoliouth 1905, p. 230; Fraser 1935: II: 25, V: 281). The best pollination results today are obtained by tying dehiscent staminate inflorescences into the pistillate inflorescences of female palms (McGregor 1976; Mbayá and Kevan 1995), or by other artificial means.

The need for caprification in Smyrna figs, i.e., providing production trees with a pollinating wasp containing caprifigs, was known in ancient Greece (Herodotus 485–425 BC) and Turkey (Condit 1920; Goor 1965). In addition, both Herodotus (485–425 BC: I: 193) and Aristotle (350 BC: 26) had some understanding of the role of wasps in pollination, although they referred to the insects involved as gall flies and psene, respectively. By the mid-eighteenth century, the process of pollination was better understood in figs according to Knuth (1909: III: 372), who reported that even Linnaeus spoke of a special “messenger of love” needed to fertilize Smyrna figs. However, this concept was vociferously ridiculed in Europe (Reasoner 1891) and California (Condit and Swingle 1947) in the mid- and late 1800s, so it is not hard to understand why the Smyrna figs introduced into California in the late 1800s failed to bear fruit. It was not until Eisen (1891) introduced the wasps into California that fruit set was achieved. However, problems persisted because of the lack of caprifig fruit; these were eventually overcome when the US Department of Agriculture implemented a program to provide such figs to growers (Rixford 1918). McGregor (1976) provides a synopsis of this story.

At about the same time as the fig pollination problem was being resolved in California, the shortage of pollinators for seed production of red clover, *Trifolium pratense*, in New Zealand prompted the introduction of bumble bees, *Bombus* spp., from Europe (Belt 1876; Dunning 1886; Hopkins 1914). Their establishment was successful, although New Zealand has still not solved its ongoing problems with regard to the pollination of kiwifruit, *Actinidia deliciosa* (Free 1993; Roubik 1995).

More recently, Malaysia, where labor costs for hand pollination are rising sharply, found a solution to its shortage of pollinators for oil palm, *Elaeis guineensis*. Syed (1979) studied the pollination of this important crop plant in its native West Africa and worked out the relationship between the pollinating weevils, *Elaeidobius* spp., and the inflorescences of the male and female palms. After careful screening and quarantine, *Elaeidobius kamerunicus* was released in Malaysian oil palm plantations, where it rapidly became established and spread (Syed et al. 1982). The result continues to be the sustainable and sufficient pollination of crops whose harvests exceed those previously produced by hand pollination, with savings of millions of US dollars per year (Kevan et al. 1986).

Another example of placing pollinators into a novel habitat to enhance crop production is the introduction of bumble bees into hothouses to pollinate tomatoes, *Lycopersicon esculentum*, in Europe (Banda and Paxton 1991) and North America (Kevan et al. 1991). Artificial pollination with electric vibrators (Kerr and Kribs 1955) is a costly method that is no longer used, whereas buzz pollination (Buchmann 1983)

by bumble bees produces superior fruit (Banda and Paxton 1991; Kevan et al. 1991). Morandin (2000) describes the efforts being made to solve the remaining technological problems related to hothouse pollination. The value of “bombiculture” for producing hothouse tomatoes and other fruits has not been assessed, but must amount to millions of dollars worldwide.

Although it may be argued that these examples are special cases and that the pollinator deficits were artificial, they serve to illustrate that when pollinator forces are insufficient, there may be inexpensive, effective alternative methods of solving problems related to pollinator deficits.

The success story of the alfalfa leaf-cutter bee (*Megachile rotundata*) and its culture for the pollination of alfalfa (*Medicago sativa*), both exotic organisms in North America, is well known. The pioneering work of Bohart (1972) and Hobbs (1967) has given rise to the multimillion-dollar industry of “megachileculture,” whose huge economic benefits are described by Olmstead and Woolen (1987). Bohart (1957) also recognized the problem of providing adequate pollination to alfalfa seed production fields, which led to the commercial development of practices for encouraging and maintaining pollinators other than honeybees, especially the alkali bee (*Nomia melanderia*). On the Canadian prairie, problems with alfalfa pollination and concomitant seed yield declines can be attributed to the expanding agriculture of the 1940s, when fields were larger and more kempt. As a result of the subsequent reduction in nesting habitat, there were too few native pollinators to provide pollination for any plants except those at the peripheries of large fields (Peck and Bolton 1946; Stephen 1955). In Manitoba, Stephen (1955) recorded yields of 1,000 kg/ha from small fields, and only 15 kg/ha from large fields. In Ontario, the contemporaneous decline of alfalfa seed production has been attributed to changing agricultural practices, including the use of insecticides.

Habitat destruction has also been a problem in the pollination of cacao (*Theobroma cacao*). Overly fastidious management of plantations included the removal of rotting vegetation, the substrate in which the pollinating midges undergo larval development (Winder 1977) and yield reductions ensued. By purposely placing appropriate plant material such as banana (Young 1982) or palm trunks (Ismail and Ibrahim 1986), adequate pollinator forces can be encouraged and maintained. The destruction of Brazilian habitat for pollinators of Brazil nuts (*Bertholletia excelsa*) has been even more detrimental to production (Maués 2001). Brazil nuts are pollinated by an assemblage of large bees whose nesting habitats have been severely curtailed or even eliminated (Sutton and Collins 1991). The vicious cycle of habitat destruction or pollution, paucity of pollinators, and failures in plant reproduction, recruitment, and regeneration has been well described (Janzen 1974; Kevan 1974, 1975a).

The catastrophic effects of recently introduced parasitic mites on honeybees have changed the face of apiculture in North America. Colony mortality and intensive management have made it more expensive to keep bees. The number of beekeepers has declined, as has the number of colonies being kept all over North America. Other pests also threaten to make beekeeping more costly and difficult. Pollination has been adversely affected and growers have reported difficulties in obtaining services

for crops such as blueberries in Maine, pome fruit in the northeastern United States and Canada, almonds in California, field cucumbers in the eastern United States and Canada, and hybrid seed production in western Canada. Economic analyses of the effects of parasitic mites are much needed for beekeeping per se and for the ancillary benefits of pollination (Morse and Calderone 2000). Although the epidemic of *Nosema* that reportedly swept through the cultures of *Bombus occidentalis* used in hothouse tomato pollination on the Pacific coast of North America has had major repercussions, the economic consequences have not been analyzed.

The adverse effects of pesticides on pollinators are well understood, especially from a toxicological viewpoint (Johansen and Mayer 1990), although less is known about their impact on crop reductions. Several works (Kevan 1975b, 1977; Kevan and LaBerge 1979; Kevan and Oppermann 1980; Kevan and Plowright 1995; Kevan 1999) explore the effects of applications of the organophosphorous pesticide Fenitrothion on nontarget habitat and on blueberry pollinators in New Brunswick, Canada. The demise of the pollinators resulted in such severe declines in the blueberry crop in the affected regions that provincial yields were significantly below those of neighboring Nova Scotia and Maine (Kevan 1977; Kevan and Oppermann 1980; Kevan and Plowright 1995), with an annual harvest loss of about 0.75×10^6 kg. Intensity of agricultural activities has also been shown to correlate with lower (by about 50 %) populations and diversity of pollinators in apple orchards in British Columbia (Scott-Dupree and Winston 1987) and berry production areas (MacKenzie and Winston 1984). Kevan (1999) present more details about these and other examples, but crop yields have rarely been included in such studies.

Although the economic impacts of pollinator declines have not been well recorded, we think it can be safely assumed that many local economies are being affected. Examples of available studies of this type include Siebert (1980), who provides an estimate of the revenue losses to both almond growers and honey producers in California resulting from a pesticide-induced decline in the numbers of pollinators; Olmstead et al. (1987), who document the historical and economic effects of the addition of pollinators on the production of alfalfa seed; and Cox et al. (1991), who show that the demise of fruit bats (Megachiroptera) through overhunting in South Pacific islands has reduced the pollination and fruit yields of some traditional harvests. No matter what their cause, would we expect anything different to result from pollinator declines elsewhere?

Although several works have attempted to illustrate the severity of pollinator declines (Buchmann and Nabhan 1996; Matheson et al. 1996; Kearns et al. 1998; Kevan 1999), the problem has generally been ignored. For this reason, it is appropriate to ask the following questions from the point of view of documentation: "Are pollinator declines real?" and "Do they have economic consequences for agriculture?" We would not only answer both questions in the affirmative, but we also believe that the problem is extremely serious, with far-reaching consequences for agriculture and global food production. However, even the most obvious example of honeybee pests and diseases should be carefully examined. The previous example of the demise of pollinators on lowbush blueberry heaths in New Brunswick shows how the basic pollination biology of crops is linked, through production agriculture and ecosystem

health, to economic impacts on consumers (Belaoussoff and Kevan 1998). Issues of scale are important. For example, even though the demise of pollinators on lowbush blueberry production fields in New Brunswick adversely affected yields, farm-gate income, and other aspects of the local economy (casual employment), it did not affect the overall market price for blueberries because that was set elsewhere by broader, regional effects (Kevan and Oppermann 1980). In the next section, we explore the broader context of interregional or international trade.

16.17 Consequences of Pollinator Decline

16.17.1 Biological Consequences

The biological consequences of pollinator decline include: less frequent flower visitation, abrupt or gradual decrease of seed and fruit production. Beekeeping sector is in danger in several areas of the world. Self-compatible flower plants can suffer from inbreeding. There is pistil senescence.

16.17.2 Economic Consequences

Economic value of a single wild bee serving as a pollinator of blueberry was estimated at US\$ 20 (Cane 1996). One hive of honeybees per hectare of apple orchards caused an increase in return equivalent to 700 % of the cost of pollination services (Kevan 1997). Value of US crops yields of pollinators other than honeybees may be as high as US\$ 6.7 billion per annum (Nabhan and Buchmann 1997).

16.17.3 The Impact of Declining Pollinator Populations on Agriculture

It is recognized that agricultural production, agroecosystem diversity, and biodiversity are threatened by declining populations of pollinators. Many pollinator population densities are being reduced below levels at which they can sustain pollination services in agroecosystems, natural ecosystems, and for the maintenance of wild plant reproductive capacity. The major contributors to this decline in pollinator populations are, inter alia, habitat loss and fragmentation, land management practices, agricultural and industrial chemicals, parasites and diseases, and the introduction of alien species. Ecological dangers of pollinator decline include the loss of essential ecosystem services (particularly agroecosystem services) and functions that pollinators provide. Ecosystem services in their turn have their own value not only biophysical, but also economical. For example, for the entire biosphere, the value

of ecological services (most of which outside the market) was estimated to be in the range of US\$ 16–54 trillion per year, with an average of US\$ 33 trillion per year (Costanza et al. 1997). Services that are provided by native pollinators (nonhoneybee species) are estimated to be worth US\$ 4.1 billion a year to US agriculture alone (Prescott-Allen and Prescott-Allen 1990). The value of the annual global contribution of pollinators to the major pollinator-dependant crops is estimated to exceed US\$ 54 billion (Kenmore and Krell 1998). In the Canadian prairies, the value of pollinators to the alfalfa seed industry has been placed at about Canadian \$ 6 million per year (Kevan and Phillips 2001).

Examples from Asia (e.g., northern Pakistan, parts of China) show linkages between declining natural insect populations and decreasing crop yields—as a result, people have begun to manage the CAB in order to maintain their crop yields and quality. For example, farmers in Himachal Pradesh (in northwestern India) are using honeybees to pollinate their apples (Partap 2003). Due to declining pollinator populations and changing cultivation practices, an increasing number of farmers around the world are now paying for pollination services and are importing and raising non-native pollinators to ensure crop production. In many developing countries, however, external pollination services are not available and rural communities have to live with reduced quantity, quality, and diversity of foods. In fruit orchards in Western China, the decline of useful insect populations has led farmers to pollinating by hand, acting as human bees. Despite a general recognition of the impact of declining pollinator populations on ecosystem functioning, and despite the examples of the ecological and economic impacts as well as examples of where this is occurring, bottlenecks and constraints hinder the conservation and management of pollinators in sustainable agriculture. An example of a globally recognized bottleneck is the lack of taxonomic information, which hampers progress that could be made not only in identifying and analyzing first pollinator populations important to agriculture, and their behavior patterns, but also best management practices. Best management practices are not readily available or known in all areas of the world, and especially not to all peoples. Indeed, a lack of awareness of pollinator issues from the farmer to the extension worker to the policy maker is also a setback for the promotion of issues related to the conservation and management of pollinators within the context of sustainable agriculture. Recognition of these bottlenecks and constraints as well as a need for action contributed to the international arena is response to the conservation and management of pollinators, in agricultural systems (and nonagricultural systems).

16.17.4 Consequences of Pollinators Loss

Due to these multiple reasons, there is an effect on pollinators and thereby on pollination. In temperate countries, several authors indicated a pollination deficit and some of them have shown that beyond an impact on agricultural productivity, the decline of pollinators affects producers in the importing countries as well as in the countries exporting agricultural products (Kevan 2001). In tropical agroecosystems, different

cases of low yields linked to inadequate pollinator services were documented, as it appears on Table 16.3 (Richards 2001). A lack of pollination can be the consequence of the introduction of an exotic plant in an area where no efficient pollinator exists. This was the case of oil palm, which was introduced in Malaysia. It was first thought the plant was wind pollinated. The fruit set being low, the pollination was carefully studied. In 1979, the pollination agent, a coleoptera, was discovered. After an analysis of the possible impact of this insect, it was introduced in Malaysia where it took the place of hand pollination of oil palm tree and to increase the yield of this new crop (Syed et al. 1982).

The introduction of Cashew nut from Brazil (native habitat) to North Borneo suffered a similar crop failure due to the lack of adequate pollinator (Allen-Wardell et al. 1998). In Southeast Asia, only few researchers studied the consequences of pollination deficit. Thus, for crops such as longan (*Euphoria longan*) and chayote (*Sechium edule*), it is stated that fields in areas with no surrounding trees (and hence no stingless bees) did not bear fruit (Heard 1999). From a wider point of view, the pollinator loss and pollination lack may have an effect at the community level. Beyond the decrease of the pollination service, the loss of a pollinator may extend to predators of this animal and, consequently on biodiversity. Food stability, with the decrease of the yield of a crop, depends on pollination. Thus, the case was documented in Maoxian County (China), where the production of apple and pear decreased. The main reason was a pollination deficiency, linked to an overuse of pesticides (Partap 2003). Whatever the causes are, the main impacts of natural pollination deficiencies are a decrease of the yield and quality of agricultural production and a possible threat on biodiversity.

16.17.5 *The Economics of Pollinators*

The inadequacy of pollinator forces for agricultural production can be offset by providing services through imported pollinators, encouraging local populations to grow, or both. However, cost-benefit analyses for pollination services in agriculture are not readily available. Some cropping systems that would lend themselves well to such studies are alfalfa seed production, hothouse tomato production, some small berry and tender fruit crops, field cucumbers and melons, almonds and other orchard crops, and specialty production systems (e.g., hybrid and horticultural seed production).

The value of pollination to alfalfa seed growers in the Canadian prairies is estimated to be 35 % of annual crop production (Blawat and Fingler 1994), although the usual practice there is for seed growers and custom pollination providers or “megachileculturalists” to share the risks, costs, and benefits in various ways. In Saskatchewan, Manitoba, and Alberta, about 30,000 ha of pedigree alfalfa seed was grown each year in 1999 and 2000, with yields of about 200–800 ha/kg worth Canadian \$ 0.50–0.75/kg. Based on a calculation of area (30,000 ha) $\times$ yield (300 kg/ha) $\times$ value (Canadian \$ 0.60 kg) $\times$ 35 %, the value per year of pollination services to the production of pedigree alfalfa seed in these provinces amounted to

Table 16.3 Examples of poor yield of crop resulting from inadequate pollinator service

Crop species	Pollinator	Reported environmental cause	Effect on yield	Reference
<i>Species requiring pollination to set seed</i>				
<i>Elais guineensis</i> Oil palm	<i>Elaeidobius kamerunicus</i>	Absence of pollinator when crop introduced to Malaysia	Almost no yield in absence of pollinator ^a	Syed 1979
<i>Averrhoa carambola</i> Starfruit	<i>A. cerana</i> , <i>Trigona thoracica</i>	Shortage of native <i>Trigona</i> pollinators in intensive orchards	Introduction of <i>Apis</i> increased fruit set	Phoon 1984, in Richards 2001
<i>Passiflora</i> spp. Passionfruit	<i>Xylocopa</i> , <i>Centris</i> , <i>Eulaema</i>	Absence of suitable nest sites, which can be provided by log piles, etc.	<i>Apis</i> and other visitors do not pollinate flowers. Hand pollination usual in commercial production	Mardan et al. 1991
<i>Durio zibetinus</i> Durian	Bats	Shortage of bats; can be improved by provision of roost sites	Self-incompatible strains will not set fruit except when bat-visited in multiclonal orchards	Vogel and Westerkamp 1991, in Richards 2001
<i>Theobroma cacao</i> Cocoa	Ceratopogonid midges	Shortage of breeding sites in pools of water (mosquito risk)	Pollination usually inadequate and supplemented by hand pollination	Young 1986, in Richards 2001
<i>Species in which seed set is increased after animal pollination</i>				
<i>Cocos nucifera</i> Coconut palm	<i>Apis</i> , <i>Melipona</i>	Absence of pollinators in plantation monoculture	Some wind pollination, but visits by stingless and honeybees increase fruit set	McGregor 1976, in Richards 2001
<i>Species in which the quality of seeds or fruits is increased after animal pollination</i>				
<i>Helianthus annuus</i> Sunflower	Various	Intensive systems reduce numbers of pollinators	Seed quality and oil yield improved after cross-pollination	Free 1993, Bichee and Sharma 1988
<i>Fragaria ananassa</i> Strawberry	<i>Apis</i> , <i>Trigona</i> , <i>Diptera</i>	Crop raised under glass	To maximize “fruit” size, 10 <i>Apis</i> visits or 30 <i>trigona</i> visits needed	Kakutani et al. 1993

^a An implication of this decline of natural pollinators is the need for managing pollination

about Canadian \$ 2 million. Pedigree alfalfa seed is grown on about one-third of the total acreage under alfalfa seed production, but common seed has a lower market value. It is reasonable to place the value of pollinators to the alfalfa seed industry at about Canadian \$ 6 million per year in the Canadian prairies, whereas the value of megachileculture (including bees for export, specialized equipment, etc.) in Saskatchewan alone, which has about half its prairie acreage in alfalfa seed production, is estimated at Canadian \$ 10 million (W. Goertzen, personal communication). Olmstead and Woolen (1987) estimated that, when pollination services were provided to Utah over the period they were studying, the increase in alfalfa production amounted to about a 600 % return on investment. These values are representative of the scale of the value of pollination, although a detailed economic analysis based on the different farming systems (e.g., dryland vs. irrigation) has yet to be carried out.

When studying apple production in Ontario, Kevan (1997) calculated roughly that providing about one hive of honeybees per hectare resulted in about one extra seed per apple, which produced larger and more symmetrical apples. These improved apples were estimated to provide marginal returns of about 5–6 %, or about Canadian \$ 250/ha, compared with an orchard without honeybees. The cost of pollination services at that time was about 1 % of production costs, and the greater yield represented a return to the grower of 700 % of the cost of pollination services. Cane (1996) assessed the value of individual wild bees (*Habropoda laboriosa*) as pollinators of rabbiteye blueberry (*Vaccinium ashei*) at about US\$ 20.00. These models represent valuable and practical approaches to evaluating pollinators as an agricultural production cost with huge potential benefits. Unfortunately, the economics of bombiculture and hothouse tomato production seem to have been set artificially by the high cost of the alternative of hand pollination.

16.18 Impact of Pollinators on World Crops

The economic impact of insect pollination on worldwide basis amounted to € 149 billion or 21.3 % of the total value of the crops that produce fruits or seeds for direct human use. The total value of crops used for human food is € 1.65 billion and 10 of the 20 most important crops worldwide depend to some extent on insect pollinators. Among 99 % of the total world crop values, 54 % rest upon crops that depends on insect pollinators. Average value of crops that depends on insect pollinators is much higher than that of the crops not pollinated by insects such as cereals and sugarcane (Rs. 50,160, and Rs. 9,900/t, respectively). The pollinator deficits may increase the cost of production as the cost of providing pollinator services rises (Anonymous 2008).

According to an estimate, the world production value for crops used for human food was € 1,618 trillion and the total value of the 46 insect-pollinated direct crops was € 625 billion, that is 39 % of the world production value during 2005. The economic value of insect pollination (EVIP) was € 153 billion. The rate of vulnerability of the world agricultural production used for human food in the face of total pollinator

Table 16.4 Economic impact of insect pollination of the world agricultural production used directly for human food and listed by the main categories ranked by their rate of vulnerability to pollination

Crop category	Average value of a production unit	Total production economic value (EV)	Insect pollination economic value (IPEV)	Rate of vulnerability (IPEV/EV)
	€ per metric ton (t)	10 ⁹ €	10 ⁹ €	%
Stimulant crops	1,225	19	7.00	39.00
Nuts	1,269	13	4.20	31.00
Fruits	452	219	50.60	23.10
Edible oil crops	385	240	39.00	16.30
Vegetables	468	418	50.90	12.20
Pulses	515	24	1.00	4.30
Spices	1,003	7	0.20	2.70
Cereals	129	212	0.00	0.00
Sugar crops	177	268	0.00	0.00
Roots and tubers	137	98	0.00	0.00
All categories pooled together	–	1,618	152.90	9.50

loss was 9.5 %. Furthermore, some regions are specialized in the production of some pollinator-dependent crop category, the vulnerability of the world production for these categories is much higher than the overall worldwide value (Table 16.3, 16.4, 16.5, and 16.6). The potential loss in some food production may not have measurable consequences in economic terms only, as it might also have serious consequences on human health. In particular, the decrease of fruit and vegetable availability could impact the health of consumers worldwide. The World Health Organization (WHO) has set a lower limit of 400 g per capita and per day for fruit and vegetable consumption (WHO Report 1985). Naska et al. (2000) studied fruit and vegetable consumption among ten European countries and found that more than 50 % of the households were below this recommendation. In the case of a total disappearance of pollinators, this situation is very likely to worsen.

16.19 Pollinator Decline: Indian Scenario

Agriculture increasingly replaces natural plant communities with monocultures, some of which are incapable of sustaining pollinator populations. Area under agriculture has increased from 97.32 million/ha in 1950–1951 to 122.83 million/ha in 2008–2009 (Anonymous 2010). Undocumented acreages of hedgerows, field margins, embankments, and other “waste places” provide nesting habitat for some native bees. Removal of these often unappreciated habitats has been associated with dramatic declines in native bee fauna. In India, urban population has increased to almost five times during the last 50 years. Increasing urbanization has resulted in converting rich arrays of habitats into highways, houses, strip malls, office complexes, and industrial parks. Urbanization not only removes habitat directly, but also isolates

Table 16.5 Geographical distribution of crop production value, economic impact of pollinators, and vulnerability ratio among the 16 subregions of the world

Geographical region and subregion	Total production EV	IPEV	Rate of vulnerability (IPEV/EV)
	10 ⁹ €	10 ⁹ €	%
<i>Africa</i>			
Central Africa	10.10	0.70	7
East Africa	19.60	0.90	5
North Africa	39.70	4.20	11
South Africa	19.20	1.10	6
West Africa	48.90	5.00	10
<i>Asia</i>			
Central Asia	11.80	1.70	14
East Asia	418.40	51.50	12
Middle East Asia	63.50	9.30	15
Oceania	18.80	1.30	7
South Asia	219.40	14.00	6
Southeast Asia	167.90	11.60	7
<i>Europe</i>			
European Union (25 members)	148.90	14.20	10
Non- European Union (25)	67.80	7.80	12
<i>North America</i>			
Bermuda, Canada, and United States	125.70	14.40	11
<i>South and Central America</i>			
Central America and Caribbean	51.10	3.50	7
South America	187.70	11.60	6

EV economic value, IPEV insect pollination economic value

and fragments the land that it does not degrade or assimilate. The attributes, extent, and permanence of fragmentation effects for native bee faunas and their flowers, however, are barely understood. Area under forest has declined and the forest land has been diverted for nonforestry purposes. As against the world average of 0.64 ha of forests per human, an Indian has only 0.06.

India, despite the rapid transition in its economy, is still predominantly an agrarian country. In India, 50 million hectare of area is under entomophilous crops, cross-pollinated by different abiotic and biotic agents. The bees earn about Rs. 10 million to the national exchequer in terms of honey production and beeswax and it is expected that an additional crop yield worth Rs. 90 million could be obtained due to pollination of crops. Considering the fact that there are indications of differing pollinator decline impacts between developing and developed world, the same requires closer scrutiny for a developing country like India, which has undergone over four decades of pesticide-dependent agricultural intensification and is also undergoing

Table 16.6 Regional effect of pollinator loss on the capacity to meet consumption before and after pollinator loss for the three crop categories for which the 2005 overall balance was negative following pollinator loss

Geographical region	Fruits		Stimulant crops		Vegetables	
	Before	After	Before	After	Before	After
<i>Africa</i>						
Central Africa	22	16	633	102	− 4	− 14
East Africa	− 51	54	300	107	6	4
North Africa	15	− 4	− 100	− 100	16	− 13
South Africa	56	29	10	− 5	2	− 14
West Africa	− 22	− 28	2,982	169	21	16
<i>Asia</i>						
Central Asia	31	− 22	− 100	− 100	40	5
East Asia	1	− 26	− 30	− 30	28	− 4
Middle East Asia	31	2	− 43	− 43	31	− 12
Oceania	60	21	− 53	− 100	− 4	− 22
South Asia	20	− 3	23	45	9	6
Southeast Asia	30	20	288	140	18	4
<i>Europe</i>						
European Union (25 members)	− 20	− 40	− 100	− 100	− 3	− 16
Non- European Union (25)	− 30	51	− 98	98	− 2	22
<i>North America</i>						
Bermuda, Canada, and United States	− 30	− 45	− 100	− 100	− 3	− 16
<i>South and Central America</i>						
Central America and Caribbean	54	35	183	99	63	22
South America	83	65	176	103	17	− 4

fast change in land use due to urbanization and industrialization. The decline in pollinator population and diversity presents a serious threat to agricultural production and conservation and maintenance of biodiversity in many parts of the country. One indicator of the decline in natural insect pollinators is decreasing crop yields and quality despite necessary agronomic inputs. Examples can be found in Himachal Pradesh in northwest India, where despite all agronomic inputs, production and quality of fruit crops, such as apples, almonds, cherries, and pears, is declining. Extreme negative impact of declining pollinator populations can be seen in other areas, for example, several farmers in Jammu and Kashmir disappointed with the very low yields and quality of apples as a result of poor pollination have chopped off their apple trees (Partap 2003).

Basu et al. (2011) assessed the long-term changes in relative yields of vegetable crops that has high level of pollinator dependency in India using 45 years' (1963–2008) FAO data. There was significant difference in the average relative yield growth rate of pollinator-dependent and pollinator-independent crops (Table 16.7). This highlights the extreme level of socioeconomic dependence of Indian agricultural systems on pollination. The above-mentioned analysis prompts an urgent enumeration

Table 16.7 The economic value of insect pollination (EVIP; US\$ million) for pollinator-dependent vegetable crop production in India

Crop species	Total value of crops (US\$ million)	EVIP (US\$ million)	Consumer surplus loss (US\$ million)
Beans (green)	116.50	5.80	6
Chillies and peppers (green)	11.10	0.55	0.57
Cucumbers	14.30	9.30	14
Egg plant	877.90	219.40	245
Pumpknins, squash, gourds	395.40	375.60	891
Tomatoes	2,305.30	115.30	118
Total	3,720.50	726.10	1,274

of the extent of natural pollinator loss in India and its impact on different pollinator-dependent crop systems across various agroecological regions of the country. It is possible that the impact would vary across various landscape scales and in different agroecological situations and a coordinated exercise toward such estimations would be essential.

16.20 What Should Be Done Now?

16.20.1 *Educate the Public on the Importance of Pollinators*

As stated previously, humans rely totally on pollination for survival and “the management and protection of wild pollinators is an issue of paramount importance to our food supply system” (Allen-Wardell et al. 1998). The European Pollinator Initiative and the Sao Paulo Declaration on Pollinator Decline both raise the need for increased public awareness of the importance of pollinators, particularly bees, and both emphasize the value in targeting the world’s education systems (Dias et al. 1999). The landmark paper on the global pollination crisis, coauthored by 22 pollination ecologists, scientists, and resource managers and endorsed by 13 universities and international organizations, “identified the need for . . . a better focus at primary, secondary and higher education levels on how pollination services benefit society” (Allen-Wardell et al. 1998).

16.20.2 *Raise Awareness of the Pollination Crisis*

Pollination ecologists and environmental scientists around the world are well aware of the pollination crisis and “an increasing number of organizations are beginning to promote the restoration of ecological functions such as pollination” (Kremen and Ricketts 2000) to the rest of the community. Time is short, as “populations of many

native plants and their pollinators are being diminished and lost due to habitat fragmentation, degradation and loss” (Allen-Wardell et al. 1998) at an increasingly rapid rate, and “loss of pollinators from a biotic community may not be easily reversible. We do not know . . . how to remedy the loss of native pollinators, or even if such remedies are possible” (Allen-Wardell et al. 1998).

The global organizations currently tackling the pollination crisis emphasize the need to raise awareness, and the European Pollinator Initiative plan of action aims to educate land managers, farmers, and conservationists:

- Train the next generation of researchers and taxonomists.
- Support national plans for the conservation of bees and increase the awareness of governments, industry, and the public.

There are a range of options to remediate the pollination crisis but, “as with many conservation issues, the final challenge will be to gather sufficient public support to implement (pollination crisis) solutions” (Kremen and Ricketts 2000).

16.20.3 Undertake Research on Alternative Pollinators

One of the biggest hurdles in overcoming the pollination crisis is lack of knowledge, as “there have been few comprehensive studies of pollination webs” (Corbet 2000) and “a serious threat to conserving pollination systems is the paucity of verifiable scientific data on pollinator abundance or effect” (Roubik 2000). The European Pollinator Initiative identifies the need to “develop alternate species of pollinator for management” as a key element, and the International Pollinator Initiative highlights the need to “assess breeding techniques of native pollinators” before serious work can begin (Dias et al. 1999).

Management of a range of bee species is required to maintain the world’s pollination systems, particularly in agricultural areas, but management is unlikely until more is known about their taxonomy, ecology, and biology. Over most of the world, even in managed agricultural areas, the pollination ecology (i.e., which species are undertaking what proportion of the pollination) is very poorly known. In natural ecosystems, our knowledge is significantly poorer still. Without properly understanding the current situation, it is difficult to determine remedies. Other important questions, which require answers to assess and rectify the situation, include: which local bee species are suitable as pollinators in each region of the globe?

1. How effective are they as pollinators and how do they compare with pollination services provided by European honeybees?
2. Can their services be improved through management and what management techniques are necessary?
3. Can local species be translocated to other areas and still have the same pollination efficacy without further disrupting the local ecosystems?
4. Over what area do the bees forage and therefore what is their pollination radius?

5. Do the bees require roosting and/or nesting sites and can these be provided by the agriculturalist, or do they require natural areas to be set aside?
6. If supplemental feeding of colonies is required, over what area do they need to forage and how is this affected by habitat fragmentation?
7. What impact will local predators and parasites have on the bees and what impact will an artificially elevated bee population have on local food webs?

The most basic requirement to answer these questions is information on the biology of each species. Social bees require different management strategies to solitary bees; nesting and foraging sites differ markedly between species; their survival rate and longevity is in part dependent on the habitat, including agricultural habitats; and the way each species harvests nectar and the efficacy of pollen transfer is also dependent on their biology and social system (Klein et al. 2002). Research is required at all levels and efforts must be made wherever possible to “invest in the development or domestication of (non-*Apis*) alternative pollinators that can be employed when the services provided by managed honeybees are inadequate to ensure high fruit set” (Allen-Wardell et al. 1998). Wherever preliminary studies have been undertaken around the world, the situation has been found to be more complex than at first glance (Klein et al. 2002), and the situation is particularly important in Australia, where “groups of native bees that have special importance for pollination systems need to be identified and cross-checked with possible conservation threats need to be made” (Schwarz and Hogendoorn 1999).

16.20.4 Diversification of Domesticated Pollinators

Together with the hived honeybees and stingless bees, there are possibilities to manage the pollination of specific crops thanks to the rearing of selected pollinators (Abrol 1997). Different experiences of pollinators bred or augmented for pollination in Asia are documented. For example, the carpenter bee *Xylocopa latipes* is found to be an efficient pollinator of passion fruit in Southern Asia. This social bee can be reared in hives having a specific design, similar to the multiple-comb hive of the honeybee (Mardan 1995). The author also indicates “the provision of wooden logs for carpenter bee nesting should be enough to provide the right number of pollinators where these bees occur” (Mardan et al. 1991). Several mango plantations in Malaysia breed flies, which seems to be the main visitors of many cultivars. In these cases, the techniques of breeding are rudimentary, based on discarded plastic bottle or plastic bags containing a breeding substrate such as fish refuse. After dipterans have laid eggs in the substrate, the receptacles are hung on branches of mango trees (Mardan 1995).

Other examples of insects that can be managed for pollination are (Sihag 1995):

1. The carpenter bee, *Xylocopa fenestrata*, which pollinates several cucurbits in semiarid and subtropical climates and which can nest in hollow stems with soft internal pith such as Castor (*Ricinis communis*) and Sarkanda (*Arundo* sp.). The internodes of preferred length and diameter are taken by females choosing a nesting sites.

2. The megachilid species *Megachile haryanaensis* and *Chalicodoma rubripes*, which are potential pollinators of alfalfa (*Medicago sativa*). They possibly nest in “paper drinking straw” of different diameters. The straws are packed in small wooden boxes carrying a thin cotton sheet at the base and closed from one side.
3. The megachilid species *Chalicodoma lanata* and *Chalicodoma cephalotes*, which are pollinators of pigeon pea (*Cajanus cajan*), can nest in rectangular wooden blocks filled with castor stems (*Ricinis communis*). With these nesting devices, it is possible to rear these insects and hence, to manage the pollination of these crops. The services in agricultural pollination offered by ground-nesting bees are also valuable and it should be interesting to better understand the role that sensory cues, learning, and memory play in nest recognition in order to be able to manage this “neglected pollinator resource” (Cane 1997).

16.21 Level of Knowledge/Awareness

Two studies (Partap and Partap 2002; Thapa 2006) reveal that the level of knowledge about biodiversity conservation, pollination, and pollinators in Nepalese farmers are inadequate. Majority of farmers are not aware of biodiversity conservation and natural pollinators or managed pollination of crops. Farmers survey revealed that more than 90 % of the citrus farmers had no idea of pollinator and pollination of citrus and very few farmers (15 %) had local bees in hive, which is just for honey production and not for managed pollination, neither they were aware of biodiversity conservation and environment protection (ICIMOD 2003).

16.22 National Policy on Pollinators

It is clear that insects including honeybees are unquestionably the main pollinating agents for many crop plants. Their role in pollinating vast array of flowering plants and maintaining biological diversity is beyond the imagination of poor farmers, politicians, policy makers, and even scientists are in dilemma. Beekeeping is known for honey production as well as pollination services to crops, but the later has received no attention in research and development activities in Nepal. Beekeeping’s important service of pollination has not only been underplayed by the planners, government authorities, but the agriculturists have also ignored altogether.

16.23 Zonation for European and Indian Hive Bees

There were nearly 100,000 Indian hive bee colonies in India few years back. Some of these bee colonies in some areas have produced honey quite comparable to European honeybees. In some areas, Indian hive bees have proved superior to European hive

bees. Indiscriminate movement of European honeybee colonies from one state to another, particularly where beekeeping with Indian hive bees already exists in sizable proportion, should be discouraged. Zonation for development of beekeeping with Indian hive bees and European hive bees is essential to avoid extinction of indigenous species of honeybees. There are some areas, e.g., agricultural plains of northern states where natural colonies of Indian hive bees are not available, but the area has the potential for beekeeping. Such areas should be first identified for introduction of European hive bees. China has nearly 700,000 indigenous hive bee colonies and about 300,000 European hive bee colonies. The indigenous bee colonies are developed in southern China, whereas the European bee colonies developed in northern China.

16.24 Conservation and Utilization of Pollinators

Honeybees show preference to more attractive floral rewards neglecting the less attractive ones (Free 1993). When two or more species of bees compete for the same floral sources, the stronger and more competitive species displace the weaker one from the resources and geographic areas affecting crop pollination. Presence of *A. mellifera* Lin. displaced and reduced the number of *A. cerana* Fab. honeybees from the resources (Neupane 2001; Mishra 1997–1998). It has become increasingly clear that the pollination needs of a crop species varies greatly with the locality and cultivar concerned; so ideally, pollination investigations are necessary in each general locality where crop is grown. Plant species are now grown for food, or other uses, in many parts of the world far from where they originated, and sometimes in absence of their natural pollinators. In such circumstances, careful consideration should be given to import natural pollinators with the introduced plant species. In addition, increased need for hybrid seed production has often posed several pollinating problems and indeed the breeders of insect-pollinated crops should always ensure that the quality and quantity of pollen and nectar produced will attract sufficient pollinators even when competitive sources are nearby. Pollinator's distribution is not systematic; some areas are overcrowded with bees and others having practically none. Proper placement of the pollinators and even their attraction to pollinating crops is necessary for good result for ensuring effective pollination. If placed properly, honeybees worked equally well in all directions and were evenly spread in flowers (Ingram et al. 1996).

For several decades, bee researchers and beekeepers have tried to conserve pollinating insects such as honeybees providing nesting sites and good forage, and protecting them from pesticides. Managed pollination of crops that has been largely the neglected part of agriculture requires due attention to increase productivity and quality. In this regard, little work has been done on the number of bee visits per flower, or the effect of cross-visitation between cultivars in relation to fruit set on crop cultivars either dependent upon or benefited by bee pollination.

Some recommendations have been made, without support or data, on colonies per hectare and suggested placement. There is no indication given as to the relative bee

population per unit of flowers and also no relation is shown between colonies per hectare and bees per flower. Studies on the foraging preference and effect of foraging competition of different honeybee species to crop pollination should find priority in future research for different ecological regions of Nepal.

In the developed countries, insect pollination has increased considerably during the past few decades and arrangements for insect pollination are now part of standard management practices when growing many crops. For example, in the United States alone, over a million honeybee colonies are rented annually for pollination services.

With hybrid seed production, it is likely that demands for pollination will become greater still in the near future. In the developing countries such as Nepal, pollination by honeybees and other pollinators is completely neglected by everyone—policy makers, naturalists, researchers, extension workers, and farmers. As far as conservationists are concerned, they have given emphasis to large mammals, birds, and reptiles, and almost nil to insect pollinators. Honeybees have been definitely concerned for honey production and Nepal is rich in traditional knowledge of beekeeping and honey production.

More than 66 laws, 38 rules and regulations, 39 executive orders, and 39 by-laws in Nepal are of direct concern to agriculture. Out of these, 17 laws are agriculture-specific addressing agricultural issues, agricultural trades, and agro industries. Reviews of these acts and regulations in the country reveal that none of them spell out the word pollinators and their conservation. Biodiversity cannot be isolated from pollinators' diversity and, therefore, there is a need to address pollinators and their conservation issues in existing acts and regulations to take care of pollinator issue. Community members as users of local resources should be aware about the importance of wild bee conservation for environment improvement and benefit sharing.

At the present day, there is now a generally increased environmental awareness for sensible habitat management that may help pollinators likely to increase. Discovering potential pollinators, devising management techniques, and increasing their population for commercial exploitation will take several years. Research studies are needed in this direction to conserve honeybees and other natural pollinators, exploit their potentiality in crop pollination, and allow them to develop in the pollution-free environment.

16.25 Current Status and Future Pollination Needs

Following recommendations (Williams 1993) are the need:

1. Research be encouraged to determine which crops and wild flowers are bee pollinated, which bee species pollinate them best and at what densities, and the economic benefits of bee pollination per crop and per region.
2. Relevant research and development and agricultural policies be adopted to ensure adequate populations of appropriate pollinators for different crops in different regions. Particular attention should be given to develop and improve techniques

for the rearing of solitary bees and bumblebees, support bee taxonomic research, promote a thriving beekeeping industry and pollination services and advice to growers, encourage the use of native rather than exotic bees, monitor movement of commercially reared bees and the impact of *Varroa*, and investigate the impact of honeybee introductions on native bee populations.

3. Land-use policies be promoted to encourage appropriate management of agricultural, forested, seminatural, conservation, and amenity areas to improve habitats for wild and managed bees. Particular attention should be given to conserve and restore natural vegetation, minimize soil surface disturbance, which destroys nest sites, promote perennial herbaceous vegetation, bee forage seed mixtures and legumes and develop pesticides safe for bees. Concern that interspecific competition for food (pollen/nectar/oil) resources between honeybees and other bee species leading to reduced reproductive success of wild bees and loss of diversity needs to be studied.

Land management for bees and conservation of bees in agroecosystems depends on habitat management sympathetic to their survival.

16.26 Conclusion

We conclude that there is ample information to suggest the existence of pollinator declines that have affected, and are affecting, agricultural productivity. Pollinator declines have different economic impacts on producers and consumers. It is essential to recognize that pollination is not a free service, and that investment and stewardship are required to protect and sustain it. Economic assessment of agriculture productivity should account for the cost of sustaining wild and managed pollinator populations.

It is apparent from the literature that at present we do not know the net effect of anthropogenic disturbance on plant–pollinator interactions. It seems likely that activities such as habitat fragmentation, agriculture, and changes to habitats caused by introduced species will be detrimental to some native species; potentially beneficial to others; and will sometimes have subtle and counterintuitive effects (Cane and Tepedino 2001). The difficulties in understanding plant–pollinator interactions are matched by the difficulties of managing and conserving for these interactions. Conservation and management is likely to be frustrated by a lack of basic information on the reproductive ecology of individuals; detection of declines in populations and the interactions of individual populations; and the different scales at which processes operate. Plants and their pollinators are mutualists, both benefiting from the relationship. While generalist pollinators may respond better to disturbance than specialists, the isolation and reduced densities of individual plant species created in disturbed areas may result in increased pollination failure in these systems (Wilcock and Neiland 2002). Both generalist and specialist pollination syndromes are likely to be impacted by disturbance, but the impacts will be different and will require different conservation and management strategies. The demographic and genetic consequences of habitat fragmentation and land-use change are likely to be species

dependent (Cane 2001). It would be unwise to assume that pollination systems are robust enough to adapt to current rates of anthropogenic change and disturbance. With implications for essential ecological processes, potential for flow-on effects to other species and serious economic implications, the conservation and management of pollination systems requires serious attention. To conclude, in order to prevent from a pollinator crisis, following steps are suggested: (1) to diversify the suite of crop-pollinating species, (2) to provide pollinating services with non-*Apis* species, (3) to conserve seminatural habitat remnant in human-dominated landscape, and (4) to detect long-term directional trends, which require coordinated regional sampling strategy over many years.

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Chapter 17

Loss of Genetic Diversity

17.1 Introduction

Himalayan region is rich in honeybee species and genetic diversity. Amongst the different native honeybee species, *Apis cerana* is equivalent of European honeybee *Apis mellifera*, as both can be domesticated and build parallel combs. There is a great genetic variance in morphological characters of *A. cerana* and the studies reveal that *A. cerana* populations can be divided into three sub-species, namely *A. cerana cerana*, *A. cerana himalaya* and *A. cerana indica*. Of these, *A. cerana cerana* is distributed over North-West Himalayas in India, North-West Frontier Province of Pakistan and Jumla region of Nepal. *A. cerana himalaya* is found in the hills of Nepal, Uttar Pradesh, North-East Himalayas and Bhutan. *A. cerana indica* is found in plain areas and foothills of the region (Verma 1996). Similar studies carried out in China reveal the presence of five sub-species of *A. cerana*. These include *A. cerana cerana*, *A. cerana skorikovi*, *A. cerana abaensis*, *A. cerana hainanensis* and *A. cerana indica* (Zhen-Ming et al. 1992; Partap 1999). Each sub-species has further locally adapted geographic ecotypes which differ from each other in several biological and economic characters. For example, sub-species *A. cerana himalaya* can be further identified into three ecotypes that correspond to geographic distribution in (1) the Naga and Mizo hills, (2) Brahmaputra valley and Khasi hills and (3) the foothills of North-East Himalayas. Despite its economic usefulness, beekeeping with *A. cerana* is suffering from precipitous decline and is threatened with extinction throughout its range. The major threat comes from its replacement with exotic and more prolific *A. mellifera*, habitat alteration, pesticide poisoning, diseases and enemies particularly the recurrence of sacbrood virus disease and human predations, especially through honey-hunting methods. Population of *A. cerana* is declining due to the following reasons: (1) Aggressive introduction and promotion of exotic *A. mellifera* by government and non-government agencies through development interventions, (2) changes in habitat and plant biodiversity, (3) incorporation of monoculture in mountain farming systems and change in land use patterns, (4) increased pesticides use due to introduction of cash crop-based farming systems and diseases and parasites.

Increasing population pressure leads to increased exploitation of land resources and changes in land use and agricultural patterns. As pesticide use is an important

element of monoculture-based agriculture so, its use becomes more prevalent. Stationary beekeeping with *A. cerana* becomes primary prey to pesticide poisoning. For example, apple farmers in Sichuan, China and Himachal Pradesh, India use 8–10 sprays of pesticides per year for protecting their apple orchards. In Baluchistan, Pakistan, apple farmers are using 3–4 pesticide applications per year.

China made a policy decision to transform wild mountain territories into fruit orchards and designated them as economic forests. This left no escape for wild bee populations in the area, as their economic forests are being sprayed and managed for higher yields.

Indigenous honeybees (*A. cerana*, *A. laboriosa*, *A. dorsata*, *A. florea*) have co-existed through centuries and kept on going without inter-specific transfer of diseases and parasites. But, with the introduction of exotic *A. mellifera*, number of diseases such as European foul brood (EFB) and acarine were introduced in the area, which disturbed the balance and harmony of co-existence amongst bee species. *A. cerana* populations were practically diminished to the level of extinction due to the spread of Thai sacbrood virus (TSBV), but within two decades resistant populations emerged and started expanding their horizon of influence in different areas, which have been reported by different authors from India, China, Pakistan and Nepal (Reddy 1999; Ge et al. 2000; Ahmad and Partap 2000; ICIMOD 2001, respectively). *Melisococcus pluton*, a parasite of *A. mellifera* was detected in the colonies of *A. laboriosa* (Allen et al. 1990). Though *Tropilaelaps clareae* is a parasite of *A. dorsata*, it found its breeding grounds in the colonies of *A. mellifera*. Stationary colonies of *A. mellifera* in the nesting areas of *A. dorsata* keep on inoculating them, while migratory *A. mellifera* colonies keep on spreading the menace. Oriental mite (*Varroa jacobsoni*) expanded its sphere of influence throughout the world, hence crippling the managed beekeeping industry in North America, Europe and Africa.

During past decades, beekeeping was defined and understood by different quarters of development workers in the perspective of honey production, which resulted in more focus on *A. mellifera* promotion in the context of beekeeping development. Keeping this in mind, institutions planned and invested heavily in training agriculture development workers in *A. mellifera* beekeeping in order to achieve high honey production. The trained manpower from within the countries and policy planners trained from abroad with honey hype in their minds forgot this fact that bees are not honey-making machines, they are very important element in conserving biodiversity and increasing farm productivity. They were not able to appreciate this fact that *A. cerana* and other indigenous honeybees of Hindu Kush Himalayan (HKH) region have evolved in this biocomplex with all the attributes of strength against local and exotic enemies. As appreciation of planners and development workers was limited and most of the time based on European and North American research and development process, they thought that it is easy to adopt a bee from northern hemisphere for sweet revolution. Though modern technology sometimes helps in pushing forward economic round turn, but if it is not linked to greater social and biological reality, then this turn becomes fatal and ecologically backfires. This is what happened in the context of beekeeping development in the mountain areas of HKH region.

This story repeated itself in each and every country of HKH region, as the policy-making institutions of these countries are the people from plains and influenced by plain area agriculture, so they further reinforced the idea of introducing *A. mellifera* in the mountain areas. This introduction was made without knowing this fact that *A. mellifera* requires migration, intensive management practices, standardised equipment and larger foraging grounds with monoculture-based agriculture. In addition to this, *A. mellifera* is prone to diseases, parasitic mites, wasps and it is very difficult for this bee to sustain in regular-changing temperature regimes. All these interventions and attempts ultimately resulted not only in the failure of *A. mellifera* introduction in the mountain areas, but also accelerated the decline of indigenous honeybee populations.

17.2 Value of *Apis cerana* Beekeeping for Mountain People

Mountain areas are characterised by inaccessibility, fragility, marginality, diversity, niche and adaptation mechanisms (Jodha 1990). *A. cerana* beekeeping system fits well with these characteristics and supports the livelihood of mountain people. Several mountain areas are inaccessible, lack of transport and communication infrastructure; so, in these circumstances, migratory beekeeping with *A. mellifera* becomes highly expensive, vulnerable and high-risk business. Stationary beekeeping system with *A. cerana* is more suitable and fits well in mountain farming systems and processes.

Mountains are fragile and their productivity is hampered by uncertain rainfall, low fertility and lack of agriculture inputs. Cycle of negative changes keeps on hitting the mountains, which results in a non-conducive situation for *A. mellifera* beekeeping, as it requires more ideal conditions for economic returns. On the other hand, *A. cerana* keeps on going under these adverse conditions, even if everything goes wrong, colonies do not abscond and farmer does not lose anything as the bees reoccupy their hives when conditions allow them to do so.

Mountain farmers are used to live on margins, so their livestock including honeybees (*A. cerana*) also evolved itself to adjust in these circumstances. *A. cerana* proved its credibility to survive, thrive and produce in highly marginal conditions. People living on margins do not have resources and information to keep themselves up to the time and emerging demands of livelihoods. It is very difficult for deprived mountain people to cater with the survival requirements of exotic *A. mellifera*.

Borders between agro-climatic zones in mountain areas are fluid and depend upon changing weather phenomenon. These agro-climatic zones lack vastness, but are rich in biodiversity. Diversity in *A. cerana* populations in different nesting areas has been evolved from the time immemorial, which evolved to an enormous strength to catch up with the difficult mountain-specific circumstances. It is reflected in its behaviour while passing through the abrupt flash raindrops, which occur in mountain valleys without any notice when bees are on foraging spree. Its foraging behaviour has also been evolved keeping in view the diversity of floral resources. Its capacity to fight, escape and adjust with the parasites and other enemies is also commendable. Bringing

a honeybee species from far away distances and adopting it into a completely different environs is really an uphill task for poor mountain farmers.

The qualitative niche and comparative advantage of native honeybees under mountain-specific condition can be explained in terms of quality of the honey, its organic background and acceptability.

Mountain communities have adapted themselves to the harsh mountain realities. Indigenous knowledge in this respect helps them to sustain the difficulties of mountain environment. *A. cerana* beekeeping is an integral part of their indigenously evolved understanding about nature, diversity and practices. People evolved management skills with minimum labour and resources to maintain Asian hive bee culture since centuries. *A. cerana cerana* of Jumla, Nepal was introduced in low-lying Kathmandu valley and this introduction was found successfully fruitful. Jumla bees adjusted the rhythm of their life in changed seasonal environmental and nectar flow regimes.

***A. cerana* Versus *A. mellifera* Beekeeping in HKH Region** *A. cerana* beekeeping system in mountain areas is based on zero investment and zero management principles, where farmers do not have sufficient money to start investment-based beekeeping. In the forest areas, people are used to catch swarms every year and keep them in their locally designed log or wall hives. In this way, they harvest small amount of honey twice a year. On the other hand, beekeeping with *A. mellifera* requires substantial amount of money to procure standardised equipment and bee colonies, which normally is beyond the reach of poor mountain farmers. In this connection, it is important to note that beekeeping is a high-risk activity. However, *A. cerana* provides an option to a farmer to start with zero investment, which neutralises the associated risks.

As discussed earlier, beekeeping with *A. cerana* does not require a lot of management such as sugar feeding, disease control and migration. So, it is easy for an isolated farming community to practice beekeeping with this bee species on the basis of their indigenous knowledge. On the contrary, *A. mellifera* beekeeping system needs all the above-mentioned management elements, which are of high cost and require broader knowledge base and training. By encouraging beekeeping with *A. cerana*, very little development investment is required and by providing very little support to farmers objective of poverty reduction can be achieved without any risk of failure (Table 17.1).

Landless and poor farmers in remote mountain areas spot and locate bee colonies in the forest areas and own, protect and exploit them. In this way, they harvest small amount of honey without disturbing bee colonies and allowing them to produce swarms, hence conserving biodiversity. This practice of hunting *cerana* honey besides conserving bee colonies helps very poor and marginalised people to make their part of livelihoods. In other words, poverty-stricken landless farmers of mountain perform and act as custodians of biodiversity conservation.

A. cerana has proved its efficiency in pollinating mountain crops and flora as compared with exotic *A. mellifera* and serving as an engine of biodiversity conservation and productivity enhancement. This oriental hive bee species has adjusted its rhythm of pollination and nectar collection services in the mountains

Table 17.1 Comparative advantages of *Apis cerana* beekeeping over *Apis mellifera* in mountain areas

Parameters	<i>Apis cerana</i> (native bee)	<i>Apis mellifera</i> (exotic bee)
Initial investment	Very low	High
Colony management costs	Negligible	High
Risk involved	Low	High
Potential of stationary beekeeping	Highly suitable	Not suitable
Scale of beekeeping	Profitable even when operated at a small scale. It is most suitable for poor beekeepers operating in remote mountain areas	Profitable only when operated at commercial scale. It is most appropriate for commercial farmers from accessible areas
Pollination of early-flowering mountain crops	More efficient	Less suitable, colony strengths low during early in the season
Indigenous knowledge	Exists	Nil
Susceptibility to mites and predators	Resistant	Susceptible
Eco-services	High	Low

since centuries. This also proves its highest level of adaptability with the changing flowering and nectar production rhythms due to uncertain and changing climatic conditions in the mountain areas. It is further to note that insurance of pollination can only be achieved by keeping and promoting this bee species in the mountain areas. Moreover, early-flowering crops of mountain areas such as almonds, peaches, plums, etc. are pollinated more efficiently by this bee species due to its ability to work in cooler climates and longer working hours. The race of *A. mellifera*, which is imported to this region, cannot ensure the objective of achieving pollination self-sufficiency, as it is vulnerable to the harsh mountain environments, diseases, adaptability and affordability by poor farming communities. Mites such as *V. jacobsoni* and *V. destructor* have virtually crippled the beekeeping industry in the Western hemisphere, while *A. cerana* has developed the ability to survive with this mite.

Beekeeping experience and associated knowledge accumulated by the mountain societies through centuries is an asset. Leaving this information and experience aside can lead to the drastic implications in the context of beekeeping development. Training mountain communities in *A. mellifera* management is a costly exercise in terms of space and time. This becomes much more expensive while considering the amount of investment and risks associated with the introduction of *A. mellifera*.

Such decline is undesirable in terms of commercial use value, maintenance of biodiversity in natural ecosystems and productivity of farming systems. In order to reverse this trend, there is a need to adopt strategies for the conservation of *A. cerana* through development and promotion of beekeeping with native bee species. Such development strategies should include studies on biology, behaviour

and management of *A. cerana* in different eco-geographic zones. Major thrust should be on further genetic improvement through classical breeding and molecular techniques and evolving the appropriate technology based on the ecological and socio-economic conditions of the region. Zonation of beekeeping areas for *A. cerana* and *A. mellifera* appears to be the only logical solution for those species, where the latter species has already been introduced.

17.3 Pollination and Biodiversity Concerns

A. cerana is a vital component of natural ecosystem. Its decline may have serious consequences for various entomophilous plant species. This bee species shows distinct advantages over *A. mellifera* for pollination of agricultural crops. These include longer foraging hours, earlier initiation of foraging activity even at 5 °C outside environmental temperature, short flight range, low cost of colony management during earth periods, no foraging competition with other native bee species, non-*Apis* pollinators and co-evolution of this bee species with native crops, etc. (Verma 1992b). Keeping in view the advantages, promotion of beekeeping with *A. cerana* with a domestic setting appears to be essential for the maintenance of biodiversity of forest and grassland ecosystem and for enhancing the productivity of farming systems. Unfortunately, in developing countries of South and Southeast Asia, the role of bees and beekeeping as an important biological input for enhancing the yield of agricultural crops has often been underestimated. The sustainable development of agriculture in the twenty-first century will therefore necessitate reorientation of the present crop production technologies. Instead of making substantial use of physical inputs such as chemical fertilisers, biocides, irrigation, heavy machinery, etc., a shift towards biological inputs such as bee cross-pollination, biological nitrogen fixation, nutrient uptake, biotechnology, etc. will become essential to increase food productivity. Moreover, such biologically based agriculture will only have positive ecological consequences. Thus, there is a need to create awareness amongst policy makers, planners and aid agencies about promoting bees and beekeeping as an important component of present-day strategies for sustainable agriculture and rural development programme (Verma 1990a)

17.4 The Amount of *Apis cerana* Genetic Diversity

The genetic diversity of *A. mellifera* has been organised into 24 sub-species having varied economic usefulness. These sub-species are adapted to a wide range of ecological conditions and at latitude ranging from 0' at equator to latitude as high as 50°N and 30°S. So far, only four sub-species of *A. cerana* are recognised, although there may be several more because of its wide range of geographic distribution (Ruttner 1987). Our research group in ICIMOD has successfully identified genetic variance

in morphological characters of *A. cerana* and confirmed the accepted distribution of *A. cerana* sub-species in the Himalayan region. These sub-species have been named as *A. cerana cerana*, *A. cerana himalaya* and *A. cerana indica* (Verma 1992a).

According to a survey conducted by bee scientists in China, five different sub-species of *A. cerana* representing different eco-geographic zones have been identified. These include *A. cerana cerana*, *A. cerana skorikovi*, *A. cerana abaensis*, *A. cerana hainanensis* and *A. cerana indica* (Zhen-Ming et al. 1992).

Each sub-species has further locally adapted populations called ecotypes which differ from each other in several biological and economic characters. For example, we have successfully identified three ecotypes of sub-species *A. cerana himalaya* that correspond to geographic distribution in (1) the Naga and Mizo hills, (2) Brahmaputra valley and Khasi hills, and (3) the foothills of the north-east Himalayas (Singh et al. 1990). On similar lines, Chinese bee scientists have also classified sub-species *A. cerana cerana* into five ecotypes namely, Guangdong-Guangxi type, Human type, Yunnan type, North China type and Changbaishan type (Zhen-Ming et al. 1992). In some parts of HKH region, *A. cerana cerana* matches European hive bee, *A. mellifera* in commercial use value and has spectacular potentials for further genetic improvement by selective breeding and molecular research.

The Values of Conserving *Apis cerana* Biodiversity The natural products which honeybees produce and human now use are honey, royal jelly, pollen, propolis, beeswax and bee venom. These materials have been widely used as nutritional food and for medicinal and pharmacological purposes since ancient times. These natural products of beekeeping industry has both consumptive and productive use value, because these can be consumed effectively without passing through a market and at the same time these hive products can be commercially harvested for exchange in formal markets throughout the world. Every common man is well aware of these direct values of this important biological resource.

However, besides these direct values, honeybees provide non-consumptive benefits of conserving botanical resources which may far outweigh direct values when they are computed. These social insects deal with such primary function of ecosystem which involve reproduction, including pollination, gene flow, cross, fertilisation, maintenance of biodiversity, environmental forces and species that influence the acquisition of useful genetic traits in economic species and maintenance of evolutionary processes, leading to constant dynamic tension amongst the competitors in ecosystems. These non-consumptive benefits can be harnessed to maximum extent through the use of native bee species such as *A. cerana* than with exotic *A. mellifera*.

17.5 How Much Genetic Diversity Is Being Lost?

Despite its economic usefulness, biodiversity of Asian hive bee *A. cerana* is suffering precipitous decline and is threatened with extinction in its entire native habitat. For example, in Japan, beekeeping with this native bee species has been completely replaced by European honeybee *A. mellifera* and only a few beekeepers and research

institutes are raising *A. cerana* colonies (Sakai 1992). In China, out of more than 8.5 million colonies of bees kept in modern hive. Of these, 70 % are exotic *A. mellifera* (Zhen-Ming et al. 1992). Similarly, in South Korea, only 16 % beekeeping is with native *A. cerana* and remaining has been replaced by exotic *A. mellifera* (Choi 1984).

According to a survey conducted by ICIMOD (2001), unfortunately beekeeping with *A. cerana* is being replaced by *A. mellifera* at such a fast rate that populations of native *A. cerana* is declining to a level that is no longer viable. These countries include Afghanistan, Bhutan, Myanmar, Nepal, India, Bangladesh and Pakistan. A visit of some mountain areas of North-West Frontier Province of Pakistan in 1989 led by Eva Crane to conclude that *A. cerana* populations may soon become an endangered species (Crane 1992). Thus, the existing centuries old and long-established craft of beekeeping with *A. cerana* has now almost got destroyed in its entire native habitat. *A. cerana* remains till now a forgotten and completely ignored species. Therefore, from biodiversity conservation point of view, it will be disastrous to leave this important genetic resource at its own and definitely require research and development interventions for its conservation and sustainable uses both in natural and agricultural eco-systems.

17.6 Causes and Consequences of Declining *Apis cerana* Diversity

In seeking ways to conserve genetic diversity of *A. cerana*, it is necessary to have a clear understanding of the major threats which this bee species is facing in its own native habitat. Similar to any other threatened biological resources, decline in *A. cerana* population is also being threatened by human mismanagement, misguided scientific and economic policies and faulty institutions. Major threats include the following: *Apis mellifera*, habitat alteration, pesticide poisoning, diseases and enemies and human predations.

17.6.1 Major Threat from *Apis mellifera*

Many importations of *A. mellifera* in South and Southeast Asia have proved disastrous for beekeeping with *A. cerana*. When kept sympatrically, *A. cerana* and *A. mellifera* colonies frequently robbed each other (Koeniger 1982). Another major problem is the transfer of parasites from one species to another. A parasite mite of brood and adults, *V. destructor* co-exist with *A. cerana* and causes no serious damage to this native bee species. In several countries of Asia, where both these species are kept together, the parasite has infested *A. mellifera* colonies and became a serious pest to this unadapted host, now killing thousands of colonies every year. It is now well documented that through importations of *A. mellifera*, *A. cerana* populations in its native habitat are facing serious risk of extinction.

On the other hand, also native *A. cerana* populations are threatened by pests and parasites of exotic western honeybee *A. mellifera* for which *A. cerana* is lacking resistance. For example, there are several reports in the literature that Thai sacbrood virus disease, European foul brood and possibly acarine disease jumped into *A. cerana* and other Asian bee species from *A. mellifera* in Nepal, India and other Asian countries killing large number of native bee colonies every year (Rana et al. 2004; Saville 2000; Allen et al. 1990).

Due to these afflictions, populations of *A. cerana* colonies practically reduced to the level of extinction, but through natural selections within two decades, normal population of this bee species stand restored from 10 % of surviving colonies (Reddy 1999; Ge et al. 2000; Ahmad and Partap 2000). These risk factors may vary between different habitat types, landscapes and bio-geographical region. The relative importance of these factors and in particular their combined effects on *A. cerana* genetic diversity loss are unknown. Large-scale importations and multiplications of exotic *A. mellifera* in to developing countries of South and Southeast Asia for better economic returns in terms of higher honey production has also become a myth. This bee species is now so seriously infested with parasitic mites, European foul brood, hornets/wasps/birds, wax moths that beekeeping with this exotic species require intensive treatment with chemicals to control these afflictions, which are very expensive and making this enterprise economically unviable. The intensity and the need for chemical treatment of *A. mellifera* colonies for mite, diseases and pest control reveal that beekeepers in developing countries of South and Southeast Asia with large uneducated, ignorant populations in isolated areas are using chemical prescriptions indiscriminately and thus affecting the quality of honey.

17.6.2 *Habitat Alteration*

In developing countries of South and Southeast Asia, habitat alteration (especially due to deforestation) from a highly diverse natural ecosystems to far less diverse (often monocultures) agro-ecosystems is adversely affecting native bee populations in the region. This is one of the most important threats often related to land-use changes on a regional scale that involve great reduction in the area of natural vegetation. Such habitat destruction could lead to loss of different types of flowering plants and bee flora. Scarcity of bee flora due to environmental degradation not only leads to decline in colony numbers, but also creates “stress conditions” for living bee colonies and increases their vulnerability to the pests and diseases, hunting and random population changes. Recent incidence of Thai sacbrood virus disease and European foul brood in *A. cerana* might have arisen due to the stress conditions created by environmental degradation. Destruction of forest habitat for growing agricultural and horticultural crops adversely affects the availability of floral resources because many of the staple crops such as rice, wheat, barley, potato, etc. are of little or no value to honeybees. Due to increasing dearth of bee floral resources, colonies in the spring would not be able to build their own populations rapidly and this might force them to forego

swarming or cause them cast smaller swarms that would reduce the probability of survival. In either case, the result would be an eventual decline in colony numbers. Habitat destruction greatly limit the choice of honeybees to carefully choose a particular micro-habitat to build nests and rear off-spring and thus protect itself from the attacks of predators. In the absence of dense vegetation, nest sites are often visible from a long distance and colonies are not able to defend themselves effectively from the predators and they become more prone to absconding.

17.6.3 Pesticide Poisoning of Honeybees

Beekeeping and pesticides both have become essential inputs of modern agricultural management technology. By ignoring either of two, global food production would be seriously impaired. Since the advent of synthetic pesticides several decades ago, the beekeeping industry, both in the developed and developing countries, have been incurring heavy losses. In developed countries, large-scale monoculture cultivation of crops and a high degree of mechanisation had greatly amplified the problem of honeybee poisoning by pesticides. However, in recent years, education and public relations have achieved much in reducing bee losses due to pesticide poisoning in the developed world.

In developing countries, all species of honeybees still face great risk because agricultural practices often include insecticide application carried out in ignorance of indifference to the indispensability of bees. In all the developing countries of South and Southeast Asia, a large area of land is being brought under the cultivation of high-yielding exotic cultivars of crops and along with them their pests have also become introduced either through human error, accidentally or lack of proper quantitative facilities. For the control of these pests, a large number of biocides are coming into use. Because of the lack of information, farmers in the region use blanket application without caring, as to what and how much to use and when. Unlike developed countries, there is also lack of legislation to prohibit the use of pesticides to the extent that kill bees. Integrated pest management technologies for protection of honeybees from harmful effects of broad-spectrum biocides are lacking. Such over reliance on chemical methods is adversely affecting environmental health including health hazards to human beings and decline in other non-target animal populations. Amongst the latter, honeybees because of their social behaviour, run the highest risk of pesticide poisoning.

17.6.4 Diseases and Enemies

There are frequent reports of *A. cerana* colonies being affected by nosema, virus cluster and sacbrood diseases in the HKH region. Recently, the European foul brood disease has badly affected *A. cerana* colonies in Kathmandu valley of Nepal. Amongst

the mites, *Acarapis woodi*, *V. jacobsoni*, *Neocyphalaeps*, *Tropilaelaps* sp. and *Pyemotes niferi* have been reported on *A. cerana*. Amongst these, acarine disease poses a serious problem (Verma 1987). Amongst the predators, five different species of wasps pose a serious threat to beekeeping industry in this region. However, because of its shimmering and evasive behaviour, *A. cerana* can resist the attacks of wasps better than *A. mellifera*. Two species of wax moths, *Galleria mellonella* and *Archoria grisella* are serious pests in *A. cerana* colonies as this native species of honeybee do not collect propolis to guard against the attack of moths.

In recent years, Thai sacbrood virus disease has been reported from all countries where *A. cerana* is found. In early eighties, the incidence and severity of this disease increased at such an alarming rate that more than 95 % of colonies infected in different countries were killed by this disease (Rana et al. 1986, 1987). This resulted in great economic loss to beekeeping with *A. cerana* in Asia not only in terms of honey and beeswax production, but also through adversely affected pollination services. The problem was particularly severe in the temperate region of the country where disease was more widespread than in tropical and sub-tropical region. The presence of Thai sacbrood virus in the diseased colonies of *A. cerana* in northern India was confirmed by conducting electron microscopic and serological studies (Rana et al. 1986, 1987). Different control measures recommended earlier to control the spread of sacbrood disease in *A. mellifera* were unsuccessful in *A. cerana* (Rana et al. 1986). However, about 5 % of colonies in the affected areas were resistant and escaped the attack of this disease. Detailed investigations on such colonies indicate that some mechanism of resistance to the sacbrood virus disease exists in *A. cerana*. In nature, this disease has a 5-year cycle, and after this period, about 5 % of the surviving colonies start multiplying in a normal way and normal population is then restored.

Due to the incidence to Thai sacbrood virus disease in *A. cerana* in recent years, the beekeepers in the region had no choice but to adopt beekeeping with *A. mellifera*, which is not only free from this disease, but is also giving higher economic returns to the farmers. Consequently, *A. cerana* has been completely abandoned by the farmers in the region and it has now become endangered/threatened species of mere academic interest to the researchers in conservation biology.

17.6.5 Human Predations

Beekeeping in Asian region is marked by a long history of honey-hunting methods that killed most of the bees, destroyed the brood and left no honey stores behind in the nest for consumption by bees during dearth periods. Such harmful exploitation by man resulted either in the loss of bee colonies or development of undesirable traits such as absconding or swarming colonies have the tendency to return to the same nesting site each year, they are thus subjected to further harmful exploitation as these locations are well known to people. The net result of such human predation is both temporal and spatial decline in bee populations in its native habitat (Bishop 1992).

Table 17.2 Components of population vulnerability analysis in *Apis cerana*. (Gilpin and Soule 1986)

Field	Component
Environmental perturbations (E)	Loss of habitat quantity and quality
	Exotic species: introduction of European honeybee <i>A. mellifera</i> L.
	Pathogens and parasites especially sacbrood virus infection
	Human predations: traditional honey-hunting methods
	Pesticide hazards
Population phenotype (PP)	Frequent swarming behaviour
	Robbing
	Large number of laying workers
	Inbreeding depression
Population structure and fitness (PSF)	Drift inbreeding
	Patch distribution
	Population fragmentation
	Demographic randomness
	Reduced effective population size
	Growth rate and distribution
	Stochastic and deterministic extinction

17.7 Population Vulnerability Analysis (PVA)

According to Gilpin and Soule (1986), loss of genetic diversity of species leading to its extinction is a systems phenomenon involving the interaction of processes and states. It is to be based on three interacting fields, i.e. population phenotype (PP), environment (E) and population structure and fitness (PSF). When such model is applied in relation to loss of genetic diversity in *A. cerana*, the first field (PP) includes behavioural and genetic components such as frequent swarming and robbing, production of large number of laying workers, inbreeding depression and drift inbreeding. The second field (E) is the context. It includes all abiotic and biotic factors that influence the population. In case of *A. cerana*, the loss of habitat quantity and quality as a result of rapid agricultural transformation and deforestation in the region and pesticide hazards due to their indiscriminate use are important abiotic components. The biotic components include introduction of exotic *A. mellifera*, epidemic of sacbrood virus disease and human predations as a result of traditional honey-hunting methods. PP and E together determine the third field, the PSF. This is the field in which dynamic consequences of interactions of PP and E are manifested in terms of patch distribution, population fragmentation, demographic randomness, reduced effective population size, growth rate and distribution leading to stochastic and deterministic extinction. The decay in one component can exacerbate not only itself, but also the behaviour of other components. Table 17.2 lists the components of each of these fields and interactions schematically.

17.8 Strategies for Conservation of *Apis cerana*

17.8.1 Stock Improvement of *Apis cerana*

Many of the above-mentioned sub-species/ecotypes of *A. cerana* are not economically viable at present. Therefore, selection and breeding programme of superior genotypes to produce honeybees suitable for intensive management is required. To achieve stock improvement, different *A. cerana* sub-species and ecotypes should be accumulated at a central location and superior genotypes be identified line breeding. The latter is essential because artificial insemination in *A. cerana* has unexpectedly turned out to be a difficult task due to very low volume of semen ejaculated by drones (Verma 1990b).

17.8.2 Apiary Management and Behaviour Research

During the course of evolution, *A. cerana* has developed certain behavioural characteristics such as frequent absconding and swarming, which are essential for the survival of colonies, but undesirable from beekeeping point of view. Our research group has identified lack of sufficient bee flora, excessive handling and exposure of colonies to summer sunshine and incidence of sacbrood virus disease as major cause of absconding. Colony performance index (CPI) reaches zero even a week before absconding. Management practices such as feeding sugar, provision of shade, providing queen gate at the hive entrance significantly reduce absconding. However, colonies affected with sacbrood virus disease show such a severe instinct of absconding that these may leave the hive even without a queen bee. One of the most effective ways of reducing frequent swarming is to follow selection programme against this undesirable trait and removal of newly constructed queen cells during active swarming season also help to check it considerably.

Currently, recurrence of sacbrood virus epidemic after an earlier cycle during 1982–1986 has threatened beekeeping with *A. cerana* throughout its range and is forcing beekeepers for its replacement by more *A. mellifera* profiles. Some colonies are still resistant to this disease and in the absence of any effective chemical control measures, vigorous selection programme need to be followed (Verma 1992a).

17.8.3 The Values of Conserving *Apis cerana* Biodiversity

The natural products which honeybees produce and human now use are honey, royal jelly, pollen, propolis, beeswax and bee venom. These materials have been widely used as nutritional food and for medicinal and pharmacological purposes since ancient times. These natural products of beekeeping industry have both consumptive and

productive use value because these can be consumed effectively without passing through a market and at the same time these hive products can be commercially harvested for exchange in formal markets throughout the world. Every common man is well aware of these direct values of this important biological resource.

However, besides these direct values, honeybees provide non-consumptive benefits of conserving botanical resources, which may far outweigh direct values when they are computed. These social insects deal with such primary functions of ecosystem as reproduction, including pollination, gene flow, cross-fertilisation, maintenance of biodiversity, environmental forces and species that influence the acquisition of useful genetic traits in economic species and maintenance of evolutionary processes, leading to constant dynamic tension amongst the competitors in ecosystems. These non-consumptive benefits can be harnessed to maximum extent through the use of native bee species such as *A. cerana* than with exotic *A. mellifera*.

Due to displacing of indigenous, well-adapted subspecies of *A. cerana*, we are going to lose these genetic resources. This objective is fully in accordance with the requirements of International Convention on Biological Diversity which specifically cites pollination as a key eco-system function that is threatened globally and loss of genetic diversity of *A. cerana* in its native habitat in Asia has very serious economic and ecological consequences, because losing genetic diversity of this bee species will not only add fragility to the existing eco-system, but millions of vulnerable farmers in this region will also lose their sustainable livelihood. We, therefore, need a concept for preserving honeybee sub-species and their ecotypes as genetic resources for future demand. The preservation of biodiversity and the enhancements towards adaptation in the honeybee should not be mistaken for a mere altruistic chore. It is an approach without alternative to provide the genetic variability needed in the future.

Honeybees are kept by men, and if the breeds do not meet the need of the beekeepers they will look for others. Consequently, it is a key issue to become informed on the apicultural demands of the beekeepers with respect to the colonies they want to work with. It is also important to consider the needs of the beekeepers in the breeding programs, otherwise it will not bear any fruitful results. A multifunctional analysis of the questionnaire (which traits and their relative importance) of several hundreds of beekeepers will provide region-specific demands of the end users of colonies, which should be considered in the breeding program.

In view of the above-mentioned situation, research work on this bee species should target to the following: Recording the actual status quo distribution of *A. cerana* sub-species and ecotypes in its native habitat. Testing races/ecotypes from different region under different environmental conditions to achieve information on their general and specific environmental adaptation and suitability for beekeepers needs. Development and implementation of breeding programs to match the demands of the beekeepers, resistance to diseases and adaptation to global warming. Development of strategies and methods to preserve this endangered honeybee species and its races; analysis of genotype–environment interactions and landscape genetics. This requires a multidisciplinary approach including population genetics, molecular genetics, physiology, morphometrics and practical bee breeding, etc. to ensure a sustainable impact on the Asian honeybee populations from a quantitative and qualitative point of view. The

listed problems cannot be solved by uncoordinated or isolated activities in different countries, but need a multidisciplinary and multinational integrated approach. The preservation and improvement of Asian indigenous honeybee races is at present the most important pressing issue, which requires immediate action. At present, very little information is available on these different basic and applied biological aspects of *A. cerana* in comparison to *A. mellifera*. Such information lies scattered in the literature and requires scientific synthesis. The research work should be carried out in different ecological zones of *A. cerana* habitat. Physical survey of each zone/site along with review of existing knowledge about genetic diversity of *A. cerana* is essential. Such work also requires consent of different nodal agency of each *A. cerana* country to execute this work plan. For detailed work plan see Chap. 22.

Training and Research Centre for Asian Bees and Beekeeping For future development of beekeeping with *A. cerana* in South and Southeast Asia, a coordinated and systematic effort by establishing a training and research centre for Asian Bee and Beekeeping is essential. This centre should have a continuing internationally funded programme in beekeeping training and research with the following objectives and organisation as given below:

1. The overall objective should be to generate and deliver improved beekeeping management technology through research and training primarily on Asiatic species of honeybee that will contribute to increased production and quality of different hive products (honey, beeswax, royal jelly, propolis and venom) as well as better bee pollination service principally to the regional needs of South and Southeast Asian countries thereby ensuring a good source of income and nutritious food to rural poor communities living at or below subsistence level.
2. To assist different Government agencies, beekeeping communities and commercial enterprises to create a cadre of beekeeping experts by training them in both practical and scientific aspects of beekeeping.
3. To provide information and advisory services and also to act as a coordinating centre for international co-operation in beekeeping.
4. To assist different developing countries of this region to establish a national programme in beekeeping.

Organisation The proposed centre may have three major programmes in beekeeping:

1. Research
2. Training
3. International co-operation.

Research may be carried out in the following basic and applied areas primarily on Asiatic species of honeybee:

1. Bee biology
2. Bee pathology
3. Bee botany and pollination
4. Beekeeping technology and equipment
5. Beekeeping economics and marketing hive products
6. Apitherapy.

Training courses both in practical and scientific aspects of beekeeping may be offered for the benefit of beekeepers, beekeeping instructors and beekeeping extension personnel from the department of agriculture, forestry and rural development representing different beekeeping communities or associations, commercial enterprises and Government departments from the entire region of South and Southeast Asia.

International co-operation programme will deal with technology sharing and technology transfer for the advancement of beekeeping in this region. It will organise regional training programmes, workshops, seminars, conferences and monitoring tours. It will also act as information dissemination centre by publishing extension literature for the popularisation and promotion of beekeeping, advisory services to individual participating countries and on regional basis also.

All the above-mentioned programmes may be carried out by keeping continuing contacts with national and international agencies. Establishment of Asian Apicultural Association (AAA) is a step forward in this direction and for the establishment of training and research centre for Asian bees and beekeeping this organisation should take initiative efforts such as change in beekeeping training curriculum at college and university levels in the light of mountain specific *A. cerana* beekeeping; re-orientation of beekeeping trainers, development workers, researchers and policy makers; establishment of country-specific centres of excellence for *A. cerana* selection, management and breeding programmes to identify, multiply and disseminate more productive races of *A. cerana*. Bee-related institutions need to build consensus on the issue of conservation-based beekeeping development. It is a need of time that all the concerned organisations and institutions should have a very fruitful and output-oriented programme on *A. cerana* conservation and dissemination. Technical backstopping can be addressed in collaboration with the scientists, development workers and beekeepers of each country in the region.

Collective consensus development on the subject may be translated into the policy formulation. As all the regional countries have different sets of problems and opportunities, so policy-level issues may be addressed in country-specific arrangements. Policy-level changes require the following approach:

1. To augment and establish viable mountain-specific extension programme on *A. cerana*-based beekeeping development.
2. On the recommendations of AAA and on the basis on our own experience, HKH region requires zonation arrangements for *A. cerana* in inaccessible mountain areas, which means banning the introduction and multiplication of *A. mellifera* in such areas.
3. Re-visiting the definition of agricultural inputs and incorporating managed pollination through honeybees as an important input in agricultural husbandry.
4. Recognition of problems in marketing of bee products and promotion of *A. cerana* mountain honey as an organic product.
5. Policy-level changes in breaking the barriers and facilitating cross-border and cross-continent trade of bee products.
6. Organizing grassroot-level beekeepers' groups/associations and their capacity building and networking.

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Chapter 18

Diseases and Enemies

18.1 Introduction

The importance of honeybees to the global world economy includes their contribution not only to the honey production but also to the pollination of many major fruit crops. Honeybees are the most economically valuable pollinators in the world. In addition to producing hive products and pollinating crops such as clover and alfalfa that are used to feed cattle that produce meat and dairy, honeybees provide pollination service to one third of crops that feed the world. It is estimated that the total economic value of pollination worldwide is € 153 billion, representing 9.5 % of the value of the world agricultural output for human food in 2005 (Gallai et al. 2009). However, increased colony losses have been reported from USA, Europe and elsewhere over the recent decades (Biesmeijer et al. 2006; Cameron et al. 2011; Ghazoul 2005; Goulson et al. 2008; Pettis and Delaplane 2010; Potts et al. 2010).

The losses of honeybees, especially the colony collapse disorder (CCD), a serious malady that resulted in a loss of 50–90 % of colonies in beekeeping operations across the USA in 2007 (Cox-Foster et al. 2007; van Engelsdorp et al. 2007), pose a serious threat to the agricultural and natural ecosystems that rely on honeybees for pollination. A combination of multiple stressors including pesticide exposure, inadequate nutrition and microbial diseases has been suggested to set off a cascade of harmful effects and contribute to increased colony losses (van Engelsdorp and Meixner 2010; Ratnieks and Carreck 2010; Carreck et al. 2010).

While the exact cause of colony losses remains unidentified, a growing body of evidence has indicated that parasites and pathogens are key culprits implicated in massive disappearance/death and population declines of honeybees (Cox-Foster et al. 2007; van Engelsdorp and Meixner 2010; Ratnieks and Carreck 2010; Carreck et al. 2010; Higes et al. 2008; Higes et al. 2009).

The Asiatic honeybee (*Apis cerana*) has a long apicultural history. While it is still found in the wild, *A. cerana* is one of the few bee species that can be domesticated and used in apiculture. Compared to European honeybee *A. mellifera*, *A. cerana* has a strong ability to work in cold temperatures as low as -0.1°C that could be lethal to *A. mellifera*. In addition, *A. cerana* possesses behavioural and physiological mechanisms to defend against the ectoparasitic mite, *Varroa destructor* (Anderson

and Trueman 2000), a species that has had a catastrophic effect on the population of *A. mellifera*. Further, *A. cerana* can fly several kilometres on foraging trips, allowing this species to utilize sporadic nectar sources (Yang 2005). With these advantages, *A. cerana* is a vitally important pollinator of crops and flora in mountainous areas, and plays a critical role in enhancing biodiversity conservation (Klein et al. 2007; He and Liu 2011). In recent years, population sizes of *A. cerana* have rapidly decreased because of extensive use of pesticides, changes of plant biodiversity, loss of habitats, competition with non-native species and interspecies transfer of pathogens and parasites (Yang 2005; He and Liu 2011; Gegear et al. 2006). Consequently, there is an acute need for investigating the causes of the bee losses and for finding novel solutions for bee health problems.

The distribution and prevalence in *A. cerana* of the trypanosome relative of *Crithidia bombi*, a gut parasite of bumblebees that has been implicated in declining bumblebee populations (Gegear et al. 2006; Yourth et al. 2008) is a matter of concern. In natural systems, most disease agents are capable of infecting multiple host species and pathogenic microorganisms that utilize multiple hosts are more often implicated in emergent diseases of animals (Pedersen et al. 2005; Woolhouse et al. 2001; Ji et al. 2003). Previous studies showed that parasites and pathogens attacking honeybees could be transmitted between different host species. For instance, *Melissococcus plutonius*, a bacterium that infects *A. mellifera* larvae leading to European foulbrood disease, caused serious damage to colonies of *A. cerana* in China from 1972 to 1976 (Yang 2005). Further, viruses that are frequent in *A. mellifera* have been identified in *A. cerana* (Ai et al. 2012; Choe et al. 2012a) and different species of bumblebees (Genersch et al. 2006b; Li et al. 2011; Peng et al. 2011), as well as in eleven non-*Apis* hymenopteran species (Singh et al. 2010). On the other hand, *V. destructor* was transmitted from its original host *A. cerana* to *A. mellifera* in the middle of the twentieth century (Oldroyd 1999) and has caused catastrophic damage to *A. mellifera* since then. More recently, a microsporidian gut parasite *Nosema ceranae* that was first discovered in *A. cerana* has emerged as a potentially virulent pathogen of *A. mellifera*. *N. ceranae* has been associated with colony collapse of *A. mellifera* and recently extended its host range to bumblebees (Chen and Huang 2010; Fries 2010).

Indeed, microbes within organisms, and particularly those within the gut, have been receiving increasing attention because of the beneficial functions they can play for their hosts. These microbes can help to digest food (Hooper et al. 2002; Fraune and Bosch 2010; Guarner and Malagelada 2003), synthesize nutrients (Breznak and Brune 1994; Janson et al. 2008), degrade ingested toxin (Vorbürger et al. 2010) and provide defences against parasites (Dillon and Dillon 2004; Dillon et al. 2005). Some studies on bacteria associated with honeybees and bumblebees have been reported that include studies showing a relatively low diversity of conserved gut microbes consisting of eight phylogenetically close species or phylotypes that share $\geq 97\%$ sequence identity in 16S rRNA sequences (Gilliam et al. 1988a; Inglis et al. 1998; Koch and Schmid-Hempel 2011a, b; Martinson et al. 2011; Mohr and Tebbe 2007; Moran et al. 2012; Sabree et al. 2012; Yoshiyama and Kimura 2009).

Honeybees, like all other creatures, suffer from many diseases and are attacked by various pests, predators and enemies. Man is concerned about the diseases of honeybees for hundreds and thousands of years. Aristotle (384–322 BC) in his *Historia Animalium*, a work on the history of animals written around 2,300 years ago, described certain disorders in honeybees (English translation by Richard Cresswell (1907)). The Roman writer, Virgil, also commented on honeybee diseases some 300 years later. However, none of the earlier observations could identify the disorders with certainty. Dzierzon (1882) recognized two kinds of foulbrood diseases in honeybees. Chesnire and Cheyne (1885) started microbial investigations into disease causing organisms. Evidently, it became widely believed that presence or absence of any disease was a matter of presence or absence of pathogens and eventual disaster was thought to be certain.

In recent times, due to fast modes of transport, intended as well as unintended transport of live honeybees and their products occurs over long distances, even between continents which has further aggravated the problem of bee diseases in their distribution. Now, any problem occurring in any part of the world in no time can assume global dimensions. Honeybees, like other social insects, are usually threatened by different pathogens, ranging from bacteria, fungi and parasites to protozoa and viruses (Schmid-Hempel 1998; The Honeybee Genome Sequencing Consortium 2006), parasitic mites, wax moths, etc. because of their crowded and warm living conditions, and social interactions, such as mutual grooming and food sharing/exchange. Besides, they are attacked by numerous predatory arachnids, wasps, birds and mammals. In most instances, the appearance of disease is abrupt and instantaneous which play havoc with honeybee colonies in apiaries. The first line of defence against honeybee diseases warrants their continuous monitoring and recognition of early stages of attack/infection so that preventive measures could be initiated well in advance before any catastrophe occurs and with interference as little as possible with their natural propensities as with many forms of life where disease is detected at an early stage, control and care is much easier to achieve. If a disease has reached an advanced stage before it is detected, the care of the colony is virtually impossible, then the colonies should be destroyed. This will avoid a weak colony being robbed out and general dissemination of the problem.

Honeybee pathology of Asian honeybee species is relatively unexplored compared to the wealth of information available on the European honeybee, *Apis mellifera*. Nevertheless, honeybees of all species share some fundamental properties that are important for host–parasite co-evolution. They all live in colonies with thousands of individuals, often packed closely together, and they all have colony-level reproduction through colony fission when they swarm. Thus, they share some common features that make some understanding of honeybee pathology from the European honeybee applicable to other honeybee species. Understanding more of the pathology of Asian honeybee species, particularly the domesticated one (*Apis cerana*), is important for several reasons. With an increased awareness of the importance of pollination and biodiversity, conservation of Asian honeybee species becomes essential. Understanding how pathogens and parasites interact within and between species may be instrumental in preventing unexpected colony losses. As we have already seen

with the *Varroa destructor* mite, and possibly with the *Nosema ceranae* fungal infections, the impact of one pathogen in one honeybee species may be very different in another closely related host system. Thus, more research on pathogens from Asian honeybee species is warranted to prevent losses from disease in these bees and to identify possible threats from interspecific transmission of pathogens.

It is thought that all bees are susceptible to the known diseases of bees, but different races may have varying susceptibility. *Apis cerana* is important in South and Southeast Asia. The colonies are small, docile and an important component for sustainable livelihood in mountain areas. The oriental honeybee has inhabited the Asian continent for millions of years, and over this long period various microbial diseases, parasites and predators have found ways of exploiting bee colonies. In fact, it is now believed that the bees' high rate of absconding is an inherited behaviour characteristic which has gradually evolved as a response to pressure on colonies from heavy predation and parasitism. Pest control is one of the most important aspects of the management of *Apis cerana*. While the primitive design of log and box hives does not allow for much choice in the way of pest-control measures, the least the traditional beekeeper can do is to ensure that the environment of his hives is free from major pests, and to make it difficult for pests to invade the hives. All cracks in hive walls should be sealed; the hive entrance should be kept as small as possible to limit the ability of wasps and wax moths to enter the hive; to protect them from ant attacks, hives should be placed on stands whose legs are coated with grease or spent oil. However, colonies in simple hives cannot be manipulated effectively to control infestation of bees and brood within the hive by tree mites and microbial diseases.

18.2 Disease Incidence

Honeybees reproduce as individuals when the queen produces offspring. However, to survive, honeybees must also reproduce at the colony level. Colony-level reproduction in honeybees occurs when the colonies swarm, increasing the number of colonies by colony fission. Without colony-level reproduction, the species would perish because colonies succumb for various reasons. Honeybee colonies are super-organisms (Moritz and Southwick 1992) that consist of individual units (bees) that have no function or survival capacity taken out of their colony context. Conceptually, this can be compared to taking simple neurons in the brain out of their brain context. Individual (unintelligent) bees become a functioning (intelligent) colony when integrated. The simple (unintelligent) neurons become a thinking (at best) brain when integrated into a whole. Colony-level reproduction offers an ideal route for pathogen transmission between colonies. If any of the swarming bees carry pathogens, these are readily transmitted to a new host colony. In fact, all honeybee pathogens known from the European honeybee, *A. mellifera*, as well as pathogens that only infect the brood stages can be transferred by adult bees (Fries and Camazine 2001). Pathogen transmission as a result of colony-level reproduction can be regarded as a vertical transference of pathogens where the parental colony infects the daughter colony.

Table 18.1 Modes of pathogen transmission within and between honeybee colonies. (Fries and Camazine 2001)

	Horizontal	Vertical
Intracolony	Worker to brood, worker, or drone	Queen to daughter (worker) Queen to daughter (queen) Queen to son (drone)
Intercolony	Worker to worker or drone Drone to worker or drone Drone to worker or drone (drifting, robbing)	Swarming

Thus, there is an important route for vertical transmission for all types of pathogens in the honeybee system. This should have important consequences for the development of pathogen virulence in honeybees whether under natural or managed conditions (Fries and Camazine 2001). Although several factors influence the evolution of virulence in pathogens, the mode of transmission between hosts is crucial (Lipsitch et al. 1996). The different modes of pathogen transmission can be divided into two categories: horizontal and vertical. Horizontal transmission refers to parasite transmission between individuals of the same generation, while vertical transmission refers to that from parent to offspring (Canning 1982). When considering colonies of social insects, we can further subdivide horizontal and vertical transmission into both intracolony (between individuals within a colony) and intercolony (between individuals from different colonies) components (Table 18.1). When horizontal and vertical pathogen transmissions are compared, vertical transmission is expected to select for decreased virulence. This is because the pathogen is dependent on host reproduction for its own transmission between hosts. Thus, in this case, the goals of the host and pathogen are aligned and a benign host–parasite relationship is likely to evolve. This trade-off between virulence and mode of transmission has been tested and confirmed experimentally in several different systems (Bull et al. 1991; Herre 1993; Turner et al. 1998). Although the main mode of pathogen transmission appears to be important for moulding host–pathogen relations, the trade-off between virulence and mode of transmission is only one, although important, aspect. Where both horizontal and vertical transmission occur, as would be the case in honeybees, the expected virulence is not simply a function of which mode of transmission predominates (Lipsitch et al. 1995a). Theoretically, it is also clear that if parasites lower the fitness of the host, they will not survive unless there is also some degree of horizontal transmission (Lipsitch et al. 1995b). Nevertheless, pathogens that depend on host reproduction for their transmission are unlikely to become virulent and cause considerable host mortality.

Honeybee pathology is almost entirely focused on managed populations of the European honeybee, *A. mellifera*. Resistance to disease may very well vary between *Apis* species, but the main difference between the pathology of European bees and the Asian honeybee species is most likely that populations of the former remain mainly domesticated in large parts of the world, whereas the latter largely exist as non-managed wild populations. Where wild populations of the European bee do

exist, their health status is not well documented, but they appear to be healthier than managed populations of honeybees (Goodwin et al. 1994; Hornitzky et al. 1996; Manning et al. 2007). Because of their importance, honeybee pathogens have been intensively studied in European honeybees and much is known about the causes of disease, the symptoms pathogens produce and how they can be controlled (e.g. cf. Bailey and Ball 1991; Morse and Flottum 1997).

However, the natural history and biological details of specific pathogens are not sufficiently well understood to define host–parasite relations in epidemiological terms. In studying the prevalence and transmission of disease agents in social insects, it is obvious that one cannot limit such studies to individual bees or to individual colonies. It is also necessary to consider the intercolonial transmission of pathogens. In terms of fitness, the successful transfer of a pathogen's offspring to a new colony is a critical step in its life history. If a parasite or pathogen fails to gain a foothold in another host colony, the parasite will not increase its reproductive fitness, regardless of how prolific it has been within the individual bee or within the original host colony.

However, only a few studies have been concerned with the actual epidemiology of honeybee diseases even in European bees. It would not be surprising if non-managed populations of honeybees have a better health status as compared to honeybees kept for apicultural purposes. Apiculture will inevitably increase the local colony density with increased opportunities for horizontal transmission of pathogens between colonies through robbing and drifting behaviour. Furthermore, the shifting of material and boxes between colonies during management may also lead to increased horizontal pathogen transmission. Interestingly, local densities of *Apis dorsata* and of *Apis laboriosa* may be as high as in managed apiaries of the European honeybee, because of their tendencies to aggregate colonies in specific trees (Deodikar et al. 1977). It would be interesting to compare if the intercolonial rates of vertical versus horizontal pathogen transmission are different in colonies of honeybees that aggregate as compared to colonies that do not, such as *Apis cerana* (Table 18.1) or *Apis florea*.

Li et al. (2012) conducted a nationwide survey to determine the occurrence and prevalence of pathogens and parasites in Asian honeybee, *Apis cerana*, in China. Their study provides evidence of infections of *A. cerana* by pathogenic *deformed wing virus* (DWV), *black queen cell virus* (BQCV), *Nosema ceranae* and *C. bombi* species that have been linked to population declines of European honeybee, *A. mellifera*, and bumblebees. However, the prevalence of DWV, a virus that causes widespread infection in *A. mellifera*, was low, arguably a result of the greater ability of *A. cerana* to resist the ectoparasitic mite *Varroa destructor*, an efficient vector of DWV. Analyses of microbial communities from the digestive tract of *A. cerana* showed that *Nosema* infection could have detrimental effects on the gut microbiota. Workers infected by *N. ceranae* tended to have lower bacterial quantities, with these differences being significant for the *Bifidobacterium* and *Pasteurellaceae* bacteria groups. The results of this nationwide screening show that parasites and pathogens that have caused serious problems in European honeybees can be found in native honeybee species

kept in Asia. Environmental changes due to new agricultural practices and globalization may facilitate the spread of pathogens into new geographic areas. The foraging behaviour of pollinators that are in close geographic proximity likely have played an important role in spreading of parasites and pathogens over to new hosts. Phylogenetic analyses provide insights into the movement and population structure of these parasites, suggesting a bidirectional flow of parasites among pollinators. The presence of these parasites and pathogens may have considerable implications for an observed population decline of Asian honeybees.

18.3 Bacterial Infections

18.3.1 American Foulbrood

American foulbrood (AFB) is caused by the spore-forming gram-positive bacterium *Paenibacillus larvae* (Genersch et al. 2006a). AFB is spread worldwide in European honeybee populations and is generally regarded as the most serious of all brood diseases of this particular honeybee (Hansen and Brødsgaard 1999). AFB is also infective for *A. cerana* (Singh 1961; Chen et al. 2000); however, the validity of the only report from the field of AFB infections in *A. cerana* (Singh 1961) has been questioned (Oldroyd and Wongsiri 2006). Although both *A. mellifera* and *A. cerana* larvae are susceptible to the disease, the Asian honeybees rarely, if ever, show clinical infections—probably because they remove infected larvae before the larvae are capped (Chen et al. 2000). It is well documented that European honeybees, with the ability to detect and remove diseased brood, can be completely AFB resistant (Spivak and Gilliam 1998). There are no reports of AFB from honeybee species other than *A. mellifera* and *A. cerana*, although this may reflect a lack of investigation rather than complete host resistance or pathogen specificity. Nevertheless, AFB appears to be a problem for managed populations of the European honeybee only. This could reflect a more developed hygienic behaviour in other species as shown for *A. cerana* (Chen et al. 2000) and/or less susceptible larvae requiring higher spore doses to become infected, as also found in *A. cerana* (Chen et al. 2000). Data from AFB infections in European honeybees suggest that the virulence of AFB, being lethal at a colony level in contrast to other honeybee diseases that are shaped by evolution, could be dependent on apicultural practices, and that the pathogen could probably be maintained without causing frequent colony mortality in a natural system (Fries et al. 2006). Considering that most Asian honeybees exist as wild populations, infections of AFB with a lack of clinical symptoms may be maintained in such populations and mainly spread between colonies through vertical transmission. This could parallel the finding of AFB spores in honey from European honeybees in parts of Africa where clinical symptoms of AFB have never been detected (Hansen et al. 2003). It would be of considerable interest if AFB spores could be retrieved from Asian honeybees and tested for both their larval- and colony-level virulence, in comparison with isolates from managed European honeybees. It has been demonstrated that different isolates

of *P. larvae* differ considerably in their larval virulence, with the most virulent isolates at the larval level and actually least virulent at the colony level (Genersch et al. 2005). Hypothetically, such strains with high virulence at the larval level and low colony-level virulence could be maintained without symptoms in honeybee populations, mainly through colony-level vertical transmission.

Chen et al. (2000) in an attempt to study the susceptibility of *A. cerana* larvae to AFB (*Paenibacillus larvae larvae*), added various doses of *P. larvae larvae* spores to larval food and disease development was monitored. Results showed that 1-day-old larvae were most susceptible, next were 2-day-old larvae, while 3-day-old larvae showed no signs of disease even when fed a large dose (4.5×10^4 spores/larva). The negative correlation between susceptibility and larval age was similar to that found for *A. mellifera*. Further, at the susceptible age, *A. cerana* larvae showed higher resistance than *A. mellifera* larvae when fed the same dose of spores. The dose of spores that would cause 95 % mortality of *A. mellifera* larvae only led to 47.1 % mortality of *A. cerana* larvae of the same age. This resistance by *A. cerana* larvae apparently was not totally related to their innate immune capability. An important aspect contributing to the resistance of *A. cerana* was the fact that up to 82.2 % of inoculated larvae were removed by adult workers before the capped stage. This adult hygienic behaviour effectively decreased the level of spore contamination inside the hive. In contrast, results showed that *A. cerana* pupae were more susceptible when vegetative cells of *P. larvae larvae* were injected into the pupal haemocoel of both species of bees.

Similarly, Ho and Chen (2001) studied the susceptibility of *A. cerana* larvae to AFB, by adding various doses of *P. larvae larvae* spores to larval food and monitoring the development of disease. Results showed that 1-day-old larvae were most susceptible, next were 2-day-old larvae, while 3-day-old larvae showed no signs of disease even when fed a large dose. At the susceptible age, *A. cerana* larvae showed higher resistance than *A. mellifera* larvae when fed the same dose of spores. This resistance by *A. cerana* larvae apparently was not totally related to their innate immune capability. An important aspect contributing to the resistance of *A. cerana* was the fact that up to 82.2 % of inoculated larvae were removed by adult workers before the capped stage. This adult hygienic behaviour effectively decreased the level of spore contamination inside the hive.

Yoshiyama and Kimura (2009) assessed the complexity of bacterial communities occurring in the digestive tract of the Japanese honeybee, *Apis cerana japonica*, using histological and 16S rRNA gene sequence analyses. Both gram-positive and gram-negative bacteria were observed, and the number of gut bacteria was higher in old larvae compared with young larvae. A total of 35 clones were obtained by a culture-dependent method, and 16S rRNA gene sequence analysis revealed that the bacterial population in the gut of Japanese honeybee was diverse, including the phyla *Firmicutes*, *Actinobacteria* and alpha-, beta- and gammaproteobacteria. Further investigation by in vitro inhibition assays was carried out to determine the ability of an isolate to inhibit *Paenibacillus larvae*, the causal agent of AFB. Out of 35 isolates, 7 showed strong inhibitory activity against *P. larvae*. Most of the antagonistic bacteria belonged to *Bacillus* species, suggesting that the bacterial isolates obtained in this study appear to be potential candidates for the biological control of *P. larvae*.

Cervancia and Fajardo (2011) studied the outbreak of the AFB in a total of 130 apiaries in Philippines. Of these, 73 were found negative for AFB, 48 were sub-clinical and 9 exhibited advanced symptoms of the disease. A total of 21 presumed *Paenibacillus larvae* isolates were obtained. The disease was widely distributed in the country as a result of beekeepers exchanging beekeeping equipment and brood combs, and moving colonies to bee pastures between localities. At least one of the isolates exhibited the potential to infect the native honeybee, *Apis cerana* Fabricius, in controlled conditions.

18.3.2 European Foulbrood

European foulbrood (EFB) is caused by the bacterium *Melissococcus plutonius*, gram-positive, lanceolate cocci (Bailey 1956). EFB is widely distributed in managed populations of the European honeybee and is regionally causing more colony losses than AFB (Wilkins et al. 2007; Roetschi et al. 2008). Bacterial cells of *M. plutonius* are digested with contaminated food and multiply within the midgut of honeybee larvae where it probably competes for nutrients with the host, who eventually dies from starvation (Bailey 1983). A high density of colonies and apiaries has been shown to promote pathogen transmission. Thus, the spatial structure of the host is important for EFB transmission (Belloy et al. 2007). Not surprisingly, problems with EFB in European honeybees are reported primarily from areas with a high colony density, such as Switzerland (Roetschi et al. 2008) and the UK (Wilkins et al. 2007).

Although reports are few, EFB has been reported to cause considerable damage, both in managed colonies of *A. cerana* (Chinh 1998) and in one colony of *A. laboriosa* (Allen et al. 1990). The first report of EFB in Asian honeybees is from 1971 when the source of infection was assumed to be European honeybee colonies introduced from the USA (Diwan et al. 1971). However, when compared to isolates from European honeybees, this isolate of *M. plutonius* from *A. cerana* could be clearly distinguished using serological typing (Bailey 1974). Thus, *M. plutonius* is probably an endemic infection in Asian honeybees.

There are no reports of EFB from honeybee species other than *A. mellifera*, *A. cerana* and *A. laboriosa*. As for the case with AFB, this probably reflects a lack of investigation rather than host resistance or pathogen specificity. The finding of EFB in managed colonies of *A. cerana*, where colony density is extreme in apiaries as compared to natural conditions, and in *A. laboriosa*, where local colony density in wild populations may also be high, could reflect the pattern observed for European honeybees: symptoms of EFB are associated with high colony density.

Ken et al. (2003) introduced brood combs of *A. mellifera* infested by EFB into five *Apis cerana* colonies and found that 4 days later all of the infested broods were cleaned, and 8 days later in the *A. cerana* colonies few broods were infested by this disease, but all the newly infested broods were also cleaned out in the next 5 days, so all the five tested colonies still keep health. Three *A. mellifera* colonies that formed a comparative group were also infested by EFB and, without any treatment, died out in about 1 month.

Rana et al. (2012) found EFB infecting 6.18–18.56 % of *A. cerana* colonies and 7.88–25.33 % of the brood in various regions of Himachal Pradesh, India. The bacteria were isolated from colonies showing EFB symptoms, and characterized using bacteriological, serological and molecular tools which confirmed infection of colonies by *M. plutonius*, the causal agent of EFB. The diseased colonies were treated by feeding four doses of 10 mg of ciprofloxacin (98 % a.i.) until the disappearance of all EFB symptoms.

18.4 Fungal Infections

18.4.1 *Nosema* spp.

Based on molecular evidence, Microsporidia are now included in the cluster Fungi (Sina et al. 2005). Thus, from a taxonomic point of view, Microsporidia are highly specialized parasitic fungi. Only two microsporidians that have been characterized are known to be infective to honeybees, *Nosema apis* (Zander 1909) and *Nosema ceranae* (Fries et al. 1996a). Microsporidians are intracellular parasites with a unique mode of cell invasion. They disperse between hosts as spores which eject a polar filament protruding from the germinating spore when in the right environment, such as the midgut of honeybees in the cases of *N. apis* and *N. ceranae*. The filament must penetrate a host cell membrane into the host cell in order to inject the infective sporoplasm into the host cell cytoplasm where parasite replication, and later spore production, is initiated (Larsson 1986). *N. ceranae* from *A. cerana* was first described in samples from the Bee Institute of the Chinese Academy of Agricultural Sciences outside Beijing, China (Fries et al. 1996a). There are earlier observations of microsporidian infections in *A. cerana*, referred to as infections by *N. apis* (Singh 1975; Lian 1980; Yakobson et al. 1992); although there are size differences between the two parasites, they are difficult to differentiate using light microscopy. Thus, these observations may in fact be observations of *N. ceranae*. Cross-infection experiments using *N. ceranae* isolates from China and *N. apis* isolates from Sweden in both *A. cerana* and *A. mellifera* in China, respectively, have demonstrated that both parasites are cross infective across hosts. However, *N. ceranae* develops better in *A. mellifera* as compared to *N. apis* in *A. cerana* (Fries and Feng 1995; Fries 1997). Thus, *N. apis* infections are probably less of a threat to *A. cerana* as compared to *N. ceranae* infections in *A. mellifera*. Indeed, natural infections in the field of *N. apis* in *A. cerana* do exist, but with a much lower prevalence as compared to *N. ceranae* (Chen et al. 2009), whereas *N. ceranae* infections in *A. mellifera* have become widespread and dominant throughout the world in recent years (Higes et al. 2006; Klee et al. 2007). Fries (2010) reviewed information on *N. ceranae* infections in European honeybees, *A. mellifera*.

Nosema is a serious disease of adult honeybees. *Nosema apis*, which causes nosema disease, is found worldwide. It belongs to a unique group of spore-forming organisms known as Microspora, many of which are parasites of insects. *N. apis* is

the most common cause of adult bee infection. Worker bees, queen bees and drones are all susceptible to infection by spores which can remain viable for considerable periods of time. Nosema disease has been reported from several parts of India. Kshirsagar et al. (1974) detected nosema disease in *Apis cerana indica* colonies in Jeolikote, Uttar Pradesh. Rahman and Rahman (1994) have recorded nosema disease in *Apis mellifera* colonies in Assam. The disease causes serious losses wherever it occurs. Moffett and Lawson (1975) found that bees infected with *N. apis* consumed less oxygen than did normal bees. The infected bees also died earlier than healthy ones. Liu (1992) found that nosemosis causes degeneration of oocyte in the ovary of the queen, the ovariole sheath gets wrinkled and ooplasm granules break down into the small spheres. Anderson and Giacon (1992) found that nosemosis also reduces pollination efficiency of bees as the bees mainly collected nectar rather than pollen. Topolska et al. (1995) reported that in Poland, nosemosis was associated with bee virus infections.

The impact of *N. ceranae* infections on *A. cerana* colonies has not been well studied. The similar lifecycle compared to *N. apis*, with infections of the ventricular cells, suggests that infections are detrimental and may be a threat to profitable beekeeping with this particular honeybee (Fries et al. 1996a). If earlier reports of damaging effects and even colony losses from microsporidian infections in *A. cerana* (Singh 1975; Lian 1980) are indeed reports of *N. ceranae* infections, this parasite may need to be controlled in *A. cerana* under certain conditions. In Asian honeybees, *N. ceranae* has also been detected in *Apis koschevnikovi* using molecular tools in samples from Indonesia (GenBank accession # FJ789802). Thus, it appears likely that this parasite is also infective for other Asian honeybee species. The impact on colony fitness from these infections in Asian honeybees remains unknown. An infection by an unknown microsporidium has been reported from *A. florea* and reported to be infective for *A. mellifera* (Böttcher et al. 1973, 1974, 1975). Nothing further is known about this infection.

Chaimanee et al. (2010) detected the microsporidium *Nosema ceranae* in honeybees in Thailand for the first time. They collected and identified species of microsporidia from the European honeybee (*A. mellifera*), the cavity nesting Asian honeybee (*Apis cerana*), the dwarf Asian honeybee (*Apis florea*) and the giant Asian honeybee (*Apis dorsata*) from colonies in northern Thailand and found the frequencies of *N. ceranae* infection in native bees to be less than that of *A. mellifera*. This endoparasite has recently been reported to infect most *Apis mellifera* colonies in Europe, the USA and parts of Asia, and is suspected to have displaced the endemic endoparasite species, *Nosema apis*, from the western *A. mellifera*.

18.4.2 Chalkbrood

Chalkbrood in honeybees is caused by the heterothallic fungus, *Ascosphaera apis*. The spores are ingested by honeybee larvae with food and the fungal hyphae eventually mummify the infected pupa. For an extensive review on *A. apis* infections in

European honeybees, see Aronstein and Murray (2010). Very little is known about chalkbrood in Asian honeybees. The pathogen has been found in *A. cerana* colonies in South Korea (Camazine 1989; Gilliam et al. 1993) and is occasionally found in the same host species in China, mainly in drone brood. It appears likely that all *Apis* species can become infected with this fungus because it is less host-specific than most honeybee pathogens. Besides *Apis* spp., *Ascospaera apis* has also been isolated from the solitary bees, *Nomandia melanderi* (Rose et al. 1984) and *Xylocopa californica* (Gilliam et al. 1994). However, there are no records of this fungal infection from any other Asian honeybee species other than from *A. cerana*, and the impact of the infection in that species remains unknown.

18.5 Virus Infections

Among the honeybee pathogens, viruses pose a strong threat to honeybees (Berényi et al. 2006; Chen et al. 2006; Shen et al. 2005). The honeybee has been reported to harbour at least 18 viruses (Allen and Ball 1996; Bailey and Ball 1991), although their infection outcome may be variable in different species or strains of honeybees. Five different virus infections have so far been identified from Asian honeybees using serological tools: Apis iridescent virus (AIV), black queen cell virus (BQCV), deformed wing virus (DWV), Kashmir bee virus (KBV) and Thai sacbrood virus (TSBV) (Allen and Ball 1996). Undoubtedly, more virus infections will be detected as molecular tools using specific primers become increasingly available.

18.5.1 *Apis Iridescent Virus*

Apis iridescent virus (AIV) was first detected in dead adult worker bees of *A. cerana* in Kashmir (Bailey et al. 1976). AIV is infective for *A. mellifera* in the laboratory, yet the infection may be limited in nature to *A. cerana* (Allen and Ball 1996); other *Apis* species that occur in Asia, however, seem likely as alternative hosts (Bailey and Ball 1978). Infections with AIV may cause severe symptoms in colonies of *A. cerana*, causing infected bees to become inactive and lose the ability to fly. This results in inactive clusters of bees in front of infected hives, rendering the name ‘clustering disease’ to the phenomenon—first believed to be caused by tracheal mites (Bailey and Ball 1978). Large colonies of *A. cerana* have been observed to perish within 2 months of becoming visibly infected (Bailey and Ball 1978). Fat body tissue is most frequently infected, but a range of other tissue types become infected as well, including ovaries. The variety of tissues that become infected suggest that the infection could be transmitted via eggs, faeces, or gland secretions (Bailey and Ball 1978).

18.5.2 *Acute Bee Paralysis Virus*

Acute bee paralysis virus (ABPV) was originally discovered as an inapparent infection of honeybees in Britain in 1963. ABPV has also been found in bumblebees and is the only bee virus known to have a natural alternate host.

18.5.3 *Kashmir Bee Virus*

Kashmir bee virus (KBV) was discovered in 1974 as a contaminant in preparations of AIV from the Asian hive bee (*Apis cerana*). The host origin of KBV is more obscure. It has been detected in *A. cerana* from Kashmir, India and Papua New Guinea as well as in *A. mellifera* populations from around the world, in bumblebees from New Zealand and European wasps (*Vespa germanica*) from Australia.

The first detection of KBV was from *A. cerana* colonies in Kashmir (Bailey and Woods 1977), and serologically related strains were later found to be also infective for *A. mellifera*. As is the case with DWV, and probably all virus infections that replicate upon injection into honeybee haemolymph, KBV is also vectored by mites that feed on honeybee brood. Indeed, KBV has also been detected in *Varroa jacobsoni* from *A. cerana* (Anderson 1989). KBV infections have not been detected in any other Asian honeybee species other than *A. cerana*, but probably exist as covert infections.

18.5.4 *Black Queen Cell Virus*

Black queen cell virus (BQCV) is associated with *N. apis* infections in *A. mellifera* (Bailey et al. 1983). Thus, it appears likely that BQCV is associated with *N. ceranae* infections in Asian honeybees, although this remains to be verified. Till date, there is only one record of BQCV infections from Asian honeybees. Using serology, BQCV was detected in diseased drone brood of *A. florea* in Iran (Allen and Ball 1996).

18.5.5 *Deformed Wing Virus*

In Asian honeybees, deformed wing virus (DWV) has so far been isolated only from *A. cerana* (Allen and Ball 1996). It appears likely that this virus is also infective for all other *Apis* species because it is infective for both *Bombus pascuorum* and *Bombus terrestris* and probably for other bumblebee species as well (Genersch et al. 2006b). The recent attention to DWV infections in European honeybees is due to the devastating effects of the infections when associated with *Varroa destructor* (Bowen-Walker et al. 1999) or *Tropilaelaps mercedesae* (Forsgren et al. 2009) infestations, where the mites serve as mechanical vectors between adult honeybees and brood.

The virus also replicates in the mites (Ongus et al. 2004; Dainat et al. 2009), probably increasing vector efficacy. In Asian honeybees, where these mites are endemic, the infestations are kept at low levels by the honeybees. Thus, DWV probably occurs mostly as covert infections, as was the case in European bees before the Asian mites were introduced (Allen and Ball 1996).

DWV is probably a widespread infection, but has remained undetected in many regions until the spread of *V. jacobsoni*. The virus has been detected in *A. mellifera* from Europe, Africa, the Middle East and Asia, and in *A. cerana* from China. Reports on the occurrence of DWV in bees from several countries have been published and detailed characterization studies on ten isolates have been undertaken. Recently, DWV was detected in Nepal and Pakistan in dead adult bees and diseased brood of *A. mellifera* infested with *Tropilaelaps clareae*. However, an association between DWV and this mite has not been determined.

18.5.6 Thai Sacbrood Virus

Thai sacbrood virus (TSBV) is serologically related to sacbrood virus in *A. mellifera*, but has distinct physico-chemical properties (Bailey et al. 1982). This virus was first isolated from *A. cerana* colonies in Thailand (Bailey et al. 1982) and appears to be the most widespread and devastating virus infection known from Asian honeybees. TSBV infections have been reported to cause considerable losses of *A. cerana* colonies (Verma et al. 1990) and are found in most areas where beekeeping with this honeybee occurs (Allen and Ball 1996). TSBV infections appear to be more detrimental to *A. cerana* colonies as compared to sacbrood infections in *A. mellifera* (Anderson 1995). TSBV and the so-called Chinese sacbrood virus (CSBV), found in *A. cerana* colonies in China, are serologically identical (Allen and Ball 1996; Yan et al. 2009). Molecular data from TSBV from India (GenBank Accession # EU156753) and from CSBV from China (GenBank Accession # AF469603) suggest that they are closely related and can be regarded as different varieties of the same virus. CSBV has been reported to be a serious threat to profitable apiculture with *A. cerana* in China (Dong et al. 1984).

It has been detected in diseased brood from Nepal (1982, 1993) and from the following states in India: Allahabad (1991), Bihar (1982), Himachal Pradesh (1985, 1993), Karnataka (1994), Kashmir (1991), Kerala (1992, 1993), Maharashtra (1995), Punjab (1985), Srinagar (1986, 1987), Uttar Pradesh (1983) and West Bengal (1989). The first record of TSBV in drone brood came from Vietnam (1989). Symptoms of sacbrood have been observed in the brood of *A. dorsata* and *A. florea* in India, but the presence of TSBV was not confirmed by serology and it is not clear whether colonies of these bees serve as reservoirs of infection for managed colonies of *A. cerana*. In China sacbrood in *A. cerana* is termed 'Chinese sacbrood' but this virus is serologically indistinguishable from TSBV. Although TSBV multiplies in *A. mellifera* in the laboratory, it does not appear to cause overt disease in this species in regions where both *A. cerana* and *A. mellifera* coexist.

TSBV disease was first observed in 1976 in Thailand on *Apis cerana* causing 100 % mortality (Bailey et al. 1982). In India, this disease first appeared in parts of Meghalaya and Assam in 1978 and by 1985 it had spread to Uttar Pradesh, Punjab and neighbouring states in North India and had virtually wiped out colonies of *A. cerana indica* from these areas (Shah and Shah 1988; Phadke 1983; Jacob et al. 1992; Kshirsagar et al. 1981, 1982; Joshi and Verma 1985). TSBV spread to the entire region of Hindu Kush Himalayas: Burma, Nepal and India. More than 95 % of the colonies were killed (Rana et al. 1986), particularly in the temperate regions where this disease is most widespread.

Verma et al. (1990) and Abrol (1993) suggested that some mechanism of resistance to TSBV disease exists in *A. cerana*. In nature, this disease had a 4-year cycle and after this period, surviving colonies began to multiply in a normal way. Devanesan and Jacob (2001) studied the larval susceptibility and found that all the four larval instars of *Apis cerana indica* F. were susceptible to TSBV. One-day-old larvae were highly susceptible recording 100 % mortality closely followed by 2- and 3-day-old larvae showing 84–92 and 82–96 % mortality, respectively. Four-day-old larvae were comparatively less susceptible recording 70–74 % mortality only. Some of the visible symptoms were decreased egg-laying rate and irregular placement of eggs, decreased foraging activity and presence of idling workers inside the hive, dwindling of bee population of the colony and desertion of the infected hive by the bees.

Lien et al. (2001) studied the situation of sacbrood disease infection on *Apis cerana* beehives in some northern provinces of Vietnam in 2000. Among beehives in 6 provinces the percentage of diseased colonies in Hung yen province was highest (30.01 %), followed by 24.91 % in Son la. In other areas it ranged from 8.84 to 12.39 %. In winter-spring season the percentage of diseased colonies was almost four times as much as that in summer-autumn season (79.83 % compared to 20.17 %, respectively).

18.5.7 Chinese Sacbrood Virus

Chinese sacbrood virus (CSBV) is the pathogen of Chinese sacbrood disease, which poses a serious threat to honeybee *Apis cerana*, and tends to cause bee colony and even the whole apiary collapse. CSBV, belonging to the genus *Iflavirus*, is the most dangerous virus associated with the collapse of the Chinese honeybee *Apis cerana* colonies due to its infection to larvae and adults and high epidemic ability (Dong et al. 1986; Ongus et al. 2004; Yang et al. 1979). Larvae with sacbrood, which fail to pupate, are full of ecdysial fluid and rich in sacbrood virus (SBV) beneath their unshed skin, and the body colour of the infected larvae changes from a pearly white to a pale yellow. Shortly after death, they dry out, forming a dark brown gondola-shaped scale (Bailey 1975). CSBV infects 1–3-day-old larvae and about one third of the infected larvae die before being capped and two third die after being capped (Yan and Han 2008). CSBV may also infect the adult bee, but in this case obvious signs

of disease are lacking (Anderson and Gibbs 1989; Bailey 1969). This virus was first observed in Guangdong Province, China in 1972 and spread to the whole China and the countries of Southeast Asia (Dong et al. 1986; Zhang et al. 2001). The genome of this virus is ssRNA with four structural proteins at its capsid. CSBV is similar to SBV in physiological and biochemical characters, but different in the antigen; these two viruses had no cross infection to the Chinese honeybees (Dong et al. 1984). Analyses of the sequenced CSBV RNA fragment revealed 87.6 % homology of the nucleotide sequence and 94.6 % homology of the deduced amino acid sequence with that of the SBV (Zhang et al. 2001; Feng et al. 1999).

Sacbrood virus (SBV) is one of the most destructive honeybee viruses. The virus causes failure to pupate and death in both larvae and adult bees. Genetic analysis of SBV infected honeybees (*Apis cerana*) from five different provinces was carried out based on three nucleotide sequences; one partial structural protein coding sequence and two non-structural protein coding sequences. Sequences amplified by three specific primer pairs were aligned and compared with reference sequences deposited in the GenBank database. Sequence alignments revealed a low level of sequence variation among Korean isolates (≥ 98.6 % nucleotide identity), regardless of the genome regions studied or the geographic origins of the strains. Multiple sequence comparisons indicated that Korean SBV isolates are genetically closely related to Chinese and other Asian strains. Interestingly, the Korean SBV isolates showed a number of unique nucleotides and amino acids that had not been observed in other strains that were studied. Korean and other Asian isolates from the host *A. cerana* and the UK, European and Japanese strains from the host *Apis mellifera* showed differences in nucleotide and deduced amino acid identities. This suggests that host-specificity exists among SBV strains isolated from different species.

Different methods have been introduced in attempts to control CSBV in *A. cerana*, including the selection of resistant bee populations, clearance of the infected hives, prevention of the temperature fluctuation in the hives, enough food supply and use of some Chinese herbs, however, no effective results are obtained (Liu 2009). RNA interference (RNAi) or post-transcriptional gene silencing (PTGS) is the phenomenon whereby double-stranded RNA (dsRNA) blocks the expression of its homologous gene, and has been reported in prokaryotes, nematodes and other invertebrate animals (Geley and Muller 2004; Hannon 2002). The honeybee genome encodes for basic components of the RNAi machinery (e.g. two Dicer enzymes, RNA-induced silencing complex, RISC proteins) (The Honeybee Genome Sequencing Consortium 2006). RNAi has been applied successfully in both adult bees (Amdam et al. 2003; Schlüns and Crozier 2007) and larvae (Beye et al. 2002). Liu et al. (2010) report on prevention of CSBV infection by feeding second instar larvae of *A. cerana* with specific sequences of CSBV dsRNA. Protection of the bee larvae from CSBV by ingestion of CSBV-derived dsRNA was further demonstrated by quantitative real-time PCR (qRT-PCR) and northern blot analysis. The result provides a potential method to protect *A. cerana* from CSBV infection.

Choe et al. (2012b) investigated the prevalence and distribution of six bee viruses in 527 *Apis cerana* samples collected from five provinces in South Korea. They found that BQCV was most prevalent virus and present in 75.11 % of 446 adult bee

Table 18.2 Mites parasitizing bees and associated with the particular bee species

<i>Apis</i> species	Mites	Source
<i>A. cerana</i>	<i>Tropilaelaps clareae</i> <i>Varroa jacobsoni</i> <i>Varroa underwoodi</i>	Delfinado-Baker et al. (1989)
<i>A. koschevnikovi</i>	<i>Varroa rindereri</i> <i>Varroa jacobsoni</i>	de Guzman and Delfinado-Baker (1996)
<i>A. nigrocincta</i>	<i>Varroa underwoodi</i>	Anderson et al. 1997
<i>A. nuluensis</i>	<i>Varroa jacobsoni</i> <i>Varroa underwoodi</i>	Delfinado-Baker et al. (1989) de Guzman and Delfinado-Baker (1996)

Table 18.3 Mite pest of honeybees, host, infestation and distribution

Mite	Host <i>Apis</i>	Colony/comb infestation	Distribution
Parasitic			
<i>Varroa jacobsoni</i>	<i>A. cerana</i>	Drone brood	Asia
<i>V. destructor</i>	<i>A. cerana</i>	Drone brood	Throughout the world except Australia
<i>Tropilaelaps clareae</i>	<i>A. cerana</i>	Worker brood	Asia
<i>T. koenigerum</i>	<i>A. cerana</i>	Worker brood and queen cells	Asia
<i>Acarapis woodi</i>	<i>A. cerana</i>	Trachea of adult bees	Asia
Non-parasitic			
<i>Neocypholaelaps indica</i>	<i>A. cerana</i>	Combs and on bees	Asia
<i>N. apicola</i>	<i>A. cerana</i>	Worker brood cells	Asia

samples, followed by SBV in 30.71 %. DWV, KBV and chronic bee paralysis virus (CBPV) were present at lower levels of 8.07, 1.56 and 0.44 %, respectively. The most prevalent virus in 81 larvae samples was SBV, with an incidence of 60.49 %, followed by BQCV in 48.14 %, DWV in 6.17 % and KBV in 1.23 % of samples. CBPV infection was not detected in larvae samples, and ABPV was not present in both larvae and adult bee. Simultaneous infections with up to four viruses were also identified. Of these, infections with SBV and BQCV were most frequent in 25.61 % of samples. The distribution of these viruses varied considerably throughout the geographic regions investigated. The three provinces of Gyeongbuk, Jeonnam and Chungbuk had the highest frequency of bee viruses.

18.6 Honeybee Mites

Honeybee species are attacked by various species of mites which cause considerable damage (Tables 18.2 and 18.3). The primary problem mites are *Acarapis woodi* Rennie, *Varroa jacobsoni* Oudemans and *Tropilaelaps clareae* Delfinado and Baker which are responsible for repeated failure of beekeeping industry in several parts of the world. The ectoparasitic mite *T. clareae* causes severe damage to the brood of

Apis mellifera colonies in Southeast Asia. It has been reported to destroy 90 % of the colonies in Afghanistan. The infestation of honeybee *A. mellifera* by *Varroa jacobsoni* is causing concern to beekeeping throughout the world. In China and Japan, there has been total loss of bee colonies in many apiaries. *Varroa* attack not only causes direct damage to honeybee colonies but also indirectly increases bee susceptibility to pathogens such as bacteria and viruses. Preference of *Varroa* mites for drone brood creates severe problem for the honeybee *Apis mellifera* whenever the latter occurs in low densities for mating. Some reports of occurrence of mites from different parts of India are available. *T. koenigerum* and *T. clareae* have been infesting all four species of honeybees, *A. florea*, *A. dorsata*, *A. mellifera* and *A. cerana* in India. *Varroa jacobsoni* and *Eugarra sinhai* have also been reported to infest colonies of *Apis cerana* in India. Among all the mite species infesting honeybee colonies, *Varroa jacobsoni* is a well known destructive pest of honeybees, *A. cerana* and *A. mellifera* in most of the Asia, while *Varroa destructor* is a recent discovery in colonies of *A. mellifera* throughout the world. *Varroa destructor* was until recently thought to be a closely related mite species called *V. jacobsoni*. Both species parasitize the Asian honeybee, *A. cerana*. The mite species originally described as *V. jacobsoni* by Oudemans in 1904 is part of the same species complex, but not the same species that made the jump to *A. mellifera*. That jump, probably, first took place in the Philippines in the early 1960s. Only after *A. mellifera* were imported to the Philippines, it came into close contact with *A. cerana*. *Varroa*, a parasite of *A. cerana*, also became a parasite of *A. mellifera*. Up until 2000, scientists did not positively identify *V. destructor* as a separate species. Up to 2005, it was known that the only *Varroa* mites that can reproduce in colonies of *A. mellifera* (Western honeybee) were the Korea and Japan/Thailand genotypes of *V. destructor*. *V. jacobsoni* is a fairly benign parasite of *A. cerana*. The mite that is known to infest the *A. mellifera* colonies was found to be a different species and has been named *V. destructor*. Anderson and Trueman (2000), after studying mtDNA Co-I gene sequences and morphological characters of many populations of *V. jacobsoni* from different parts of the world, considered it to be a species complex and split it into two species. *V. jacobsoni* sensu stricto infests *A. cerana* F. in the Malaysia–Indonesia region. *V. destructor* Anderson and Trueman infests its natural host *A. cerana* on mainland Asia and also *A. mellifera* L. worldwide. Anderson and Trueman (2000) reported two haplotypes of *V. destructor* infesting *A. mellifera*, the more virulent and widespread Korean haplotype, and the less virulent Japan/Thailand haplotype. Anderson and Trueman (2000) identified two haplotypes of *V. destructor* that infest *A. cerana* in Asia and have become pests of *A. mellifera* worldwide. The Korea haplotype is the common one, being a parasite of *A. cerana* in Korea and now a pest of *A. mellifera* in Europe, the Middle East, Africa, Asia, North America and South America. The Japan/Thailand haplotype is less common, being a parasite of *A. cerana* in Japan and Thailand and also a pest of *A. mellifera* in Japan, Thailand and the Americas. The Korea haplotype of *V. destructor* appears to be more pathogenic to *A. mellifera* than the Japan/Thailand type (Anderson and Trueman 2000). The New Zealand *V. destructor* is likely to be the widespread Korea haplotype. However, its DNA sequencing is required to confirm this. *Varroa destructor* is much more widespread than *V. jacobsoni* sensu stricto, and the Korea

haplotype of *V. destructor* has the greatest geographical range among four *Varroa* species (Anderson and Trueman 2000).

The mite is devastating to beekeeping in most parts of the world. The negative effect from the mite primarily is related to virus infections where the mite triggers replication of dormant infections that become overt. The mite vectors DWV, ABPV and slow paralysis virus (SPV). Fast development of resistance in *Varroa* mites is a major impediment in evolving chemical control. Of the several management strategies, such as cultural, biological, chemical, genetic or hygienic behaviour, hygienic behaviour is a desirable trait in honeybees (*Apis mellifera* L.), as hygienic bees quickly remove diseased brood, interrupting the infectious cycle. Hygienic lines of honeybees appear to be more sensitive to the odours of dead and diseased honeybee brood.

The mites are passing their pesticide resistance to their progeny faster than scientists can develop new compounds to fight them. Honeybee populations have been decimated, which could severely affect pollination of wild flowers and other plants. Therefore, sustainable technologies are required for their management.

In honeybees, brood parasitism by parasitic mites (Acari) has evolved in every lineage except *A. mellifera* (Eickwort 1994). Despite the fact that all parasitic mites of honeybees are native to Asia, except for one tracheal mite species, we rarely encounter cases where these mites cause serious damage to their native hosts (Boot et al. 1997; Oldroyd 1999). Natural selection for benign host–parasite interactions might play a role in these relationships to allow co-existence between the bees and the mites (Fries and Camazine 2001; cf. Chap. 15). However, the more domesticated *A. mellifera* is not as fortunate as its relatives. As in many cases with horizontal transmission of a parasite to a new host, the host is ill equipped behaviourally and physiologically to defend itself and may succumb to the novel invader (Lipsitch et al. 1995a, b. Thus, *A. mellifera* falls victim to these mites of Asian origin, which frequently results in the disintegration of colonies.

In Asia, two families of parasitic mesostigmatid mites viz. Varroidae and Laelapidae are largely represented (Table 18.3). The tracheal mite, *Acarapis woodi*, in another family of parasitic mites from the order Prostigmata, is native to Europe and is considered to be an introduced parasitic species of Asian honeybees. The most well-known case of an Asian mite introduction into Europe and the Americas is the notorious *Varroa* mite that switched hosts from its native Asian cavity-nesting species, *Apis cerana* to *Apis mellifera*, which later spread to almost every part of the world (Griffith and Bowman 1981; De Jong et al. 1982; de Guzman et al. 1997, 1998; Sammataro et al. 2000; Zhang 2000). Vast resources and attention have been invested in studies of chemical and biological treatments of *Varroa* on *A. mellifera*, despite numerous reports on the problems of differential resistance of the mites to these treatments (cf. Gerson et al. 1991; Lodesani et al. 1995; Colin et al. 1997; Eischen 1998a, b; Elzen et al. 1998). However, almost a century after the discovery of *Varroa*, we learned that the variations in resistance to *Varroa* stem from the genetic variations in different populations of these superficially similar mites (Anderson and Trueman 2000). For the past decade, molecular genetics combined with classical

Table 18.4 Mesostigmatid species of parasitic honeybee mites, their native hosts and geographical distributions in Asia. (Data presented here are compiled from references cited in the text)

Mite species	Native hosts	Geographical distributions
Family Varroidae		
Genus <i>Varroa</i> Oudemans (1904)		
<i>V. destructor</i> Anderson and Trueman (2000)	Mainland <i>A. cerana</i> ^a	India, Pakistan, Nepal, China, Japan, Korea, Taiwan, northern Thailand, Vietnam
<i>V. jacobsoni</i> Oudemans (1904)	Sundaland <i>A. cerana</i> ^a	Central and southern Thailand, Malaysia, Indonesia, Palawan, Island
<i>V. underwoodi</i> Delfinado-Baker and Aggarwal (1987a)	<i>A. nigrocincta</i>	Sulawesi Island
	<i>A. cerana</i>	Presumably in <i>A. cerana</i> range except for China, Vietnam, Philippine Islands
	<i>A. nigrocincta</i> ^b	Sulawesi Island
	<i>A. nuluensis</i> ^b	Mt. Kinabalu, Borneo
<i>V. rindereri</i> de Guzman and Delfinado-Baker (1996)	<i>A. koschevnikovi</i>	Malaysia, Indonesia except islands beyond east of the Wallace's line

^a *A. cerana* populations in Asia can be categorized as belonging to four major mitochondrial lineages (Smith and Hagen 1996; Smith et al. 2000). Two of the four of these lineages have wide distributions. The so-called 'Mainland' *A. cerana* is distributed throughout the India–Pakistan sub-continent, China, Japan, Laos, Cambodia, Vietnam, north and central Thailand. The 'Sundaland' population occupies southern Thailand, Malaysia and Indonesia. The other two lineages occur in the Philippines

^b The records of *V. underwoodi* on *A. nigrocincta* and *A. nuluensis* have never been confirmed. However, the probability of finding *V. underwoodi* on these bees is high (Oldroyd and Wongsiri 2006)

taxonomic studies have shed light and revealed a lack of homogeneity in the populations of the parasitic mites of the honeybees in Asia. A list of currently described parasitic mesostigmatid mites and their native hosts in Asia is given in Table 18.4.

18.6.1 *Varroa* Mites

One of the prominent parasitic mites that have a long history with beekeepers around the world is the genus *Varroa* (Family Varroidae). The type species of the genus was described early in the twentieth century and named *V. jacobsoni* Oudemans (1904) from a colony of *A. cerana* in Java, Indonesia. *Varroa* mites later spread into *A. mellifera* colonies that were introduced into Asia and have since infected many commercial bee colonies around the world due to transcontinental shipments of bee colonies (de Guzman et al. 1997, 1998). *Varroa* has now spread into most parts of the world except for Australia and the Antarctica (Sammataro et al. 2000). A decade ago, New Zealand and Hawaii were considered to be *Varroa*-free zones; however, recent reports have shown that the mites have already become established in those areas (Zhang 2000; HDOA 2010).

Varroa mites are generally elliptical and dorsoventrally flattened, which provides the mites with the ability to attach themselves inconspicuously to sclerites of bees or to hide between areas where it is difficult for the bees to groom, such as the propodeum (Yoder et al. 1999). The colour of *Varroa* ranges from reddish brown to bright red. In an *A. cerana* colony, *Varroa* can reproduce only in the drone brood cells (Peng et al. 1987; Büchler et al. 1992; Fries et al. 1996b; Boot et al. 1997; Rath 1999) because the bees can detect, groom and remove the mites from worker bees and worker brood cells. This bee behaviour reflects a coadaptation between the host and parasite during their evolutionary history (Oldroyd 1999; Rath 1999; Sasagawa et al. 1999).

All species of the genus *Varroa* are native to Asia. Current knowledge on *Varroa* taxonomy recognizes four valid species: *V. jacobsoni* Oudemans, *V. destructor* Anderson and Trueman, *V. underwoodi* Delfinado-Baker and Aggarwal (1987a) and *V. rindereri* de Guzman and Delfinado-Baker (1996). However, at least one population in the Philippine Islands has a distinct genetic composition different from others in mainland Asia and Indonesia that may also be valid as a separate species (Anderson and Trueman 2000; Anderson 2004). Only *V. destructor* is reported to colonize and wreak havoc on *A. mellifera* (Anderson and Trueman 2000; Warrit et al. 2006; Navajas et al. 2010). *A. cerana* is the primary host to at least three species of *Varroa* in Asia, except for *V. rindereri*. As per current knowledge, we know that *A. koschevnikovi* is parasitized only with *V. rindereri* (de Guzman and Delfinado-Baker 1996). A congeneric of *A. cerana* in Sulawesi, *A. nigrocincta*, is reported to harbour *V. jacobsoni* (Anderson and Trueman 2000), while there is speculation that *V. underwoodi* may infest this bee, and also *A. nuluensis* on Mt. Kinabalu, Malaysia (Otis and Kralj 2001). *V. jacobsoni* was once believed to have as wide a distribution as *A. cerana* ranging throughout Asia; however, before 2000, there were numerous reports on pathological and genetic variations of *V. jacobsoni* in both *A. cerana* and *A. mellifera* worldwide (Moritz and Haenel 1984; Camazine 1986; Delfinado-Baker 1988; Delfinado-Baker and Houck 1989; Ritter et al. 1990; Moretto et al. 1991; Anderson 1994; Eguaras et al. 1995; Kraus and Hunt 1995; Anderson and Sukarsih 1996; De Jong and Soares 1997; de Guzman et al. 1997, 1998; Anderson and Fuchs 1998). Subsequently, Anderson and Trueman (2000) published a breakthrough paper that revealed that *V. jacobsoni* is in fact a complex of at least two species—*V. jacobsoni* sensu stricto and *V. destructor*. This finding was based on genetic variations of the cytochrome c oxidase I (COI) gene and the results of reproductive isolation experiments of both mites on *A. cerana* and *A. mellifera*. The study revealed that *V. destructor* is a native parasite of *A. cerana* in mainland Asia.

The natural distribution of *V. destructor* ends in northern Thailand (Anderson and Trueman 2000; Warrit et al. 2006), while further down the Malay Peninsula and the Indonesian archipelago, the mite that reproduces in native *A. cerana* is *V. jacobsoni*. This finding also suggested that the mite species in Asia might have co-evolved with certain *A. cerana* mtDNA lineages (Anderson and Trueman 2000; Warrit et al. 2006). Only populations of *V. destructor* from Korea and Japan (designated by Anderson and Trueman (2000) as ‘K’ and ‘J’ haplotypes) are known to reproduce successfully in *A. mellifera*. The ‘K’ haplotype was reported to be far more virulent than the ‘J’

counterpart (de Guzman et al. 1997; de Guzman and Rinderer 1999; Anderson and Trueman 2000; Garrido et al. 2003). Navajas et al. (2010) suggested that the unique genetic make-up of Japanese and Korean *V. destructor* may be a factor facilitating their colonizing of *A. mellifera* and might have stemmed from a genetic 'bottleneck' event in the *Varroa* population in mainland Asia. They also added that the 'K' and 'J' haplotypes have variants that are well established in *A. mellifera* colonies in China, Taiwan, Vietnam and Thailand that have never been reported in *A. mellifera* in Europe and the Americas and might pose a new threat to apiculture worldwide. Little is known about the basic biology of the more obscure *V. underwoodi* and *V. rindereri*. It is widely understood that *V. underwoodi* can be found with *A. cerana* throughout its range and sometimes living sympatrically with *V. destructor* or *V. jacobsoni* depending upon the locality of *A. cerana* (Oldroyd and Wongsiri 2006). With the distribution of *A. koschevnikovi* in the Malay Peninsula and the Indonesian archipelago, one can assume that *V. rindereri* is spread along with this bee in the region, except for the islands east of the Wallace' line (Otis 1996).

18.6.1.1 Life Cycle of *Varroa*

The life cycle of *V. destructor* on *A. mellifera* is well studied and can be related to an understanding of this mite on *A. cerana* (De Jong 1997; Donzé and Guerin 1994, 1997; Sammataro et al. 2000; Oldroyd and Wongsiri 2006). Life cycle of *Varroa* consists of two phases: phoretic and parasitic (Oldroyd and Wongsiri 2006). In the parasitic phase, the gravid adult female mite enters the prepupal brood cell 1 or 2 days before cell-capping by the nurse bees. The mite conceals itself submerged in the liquid brood food until the cell is capped. The peritreme structure of the mite is used like a snorkel to help the mite breathe under the liquid food (Donzé and Guerin 1997). After the cell is capped and the prepupa is formed, the mite starts feeding on the bee haemolymph. About 60 h after the cell is capped, the mother mite produces her first egg, which develops into a male. All of the subsequent eggs (usually 3 or 4), which are laid at 30-h intervals, are destined to become females. Immature mites (nymphs) feed on the haemolymph of the prepupa at the site where the mother mite usually feeds (Donzé and Guerin 1994). Male mites require slightly less time than females (5–6 and 6–7 days, respectively) to develop into adults. Mating occurs between siblings of the same brood. The life cycle of the male mite is short compared to the females. The male mite dies in the brood cell after the last female sister is fertilized and before the cell is uncapped. After the brood cell is uncapped, the female mite begins its phoretic phase. However, if the bee colony is not in its reproductive cycle, the mite will seek for a newly emerged adult bee as its short-term host. The mite stays on the bee's metasoma, or the area behind the head, and feeds continuously most of the time. The phoretic phase of the mite is shortened if the infested colony is full of new eggs that have recently been laid by the queen bee and the larvae. Le Conte et al. (1989) suggested that, because of the higher levels of fatty acid esters produced by drone larvae, the female mites prefer invading drones more than worker cells.

18.6.1.2 Impact of *Varroa* on *A. cerana*

Tewarson et al. (1992) provided the first insights into the life cycle of *V. destructor* on an *A. cerana* colony in India. The mite population growth rate in an *A. cerana* colony is significantly slower than in *A. mellifera* (Boot et al. 1997). Most of the time, the mites infest the drone brood, and it is rare to observe the mites invading worker cells. Thus, the damage to the colony of the mite's native host is not as severe as we observe in *A. mellifera*, where the mites are found reproducing successfully in worker cells. The defensive behaviours of *A. cerana* against the mites can explain the low growth rate of the mite population. The worker bees can groom each other to remove mites, a behaviour that is lacking in *A. mellifera* (Peng et al. 1987; Büchler et al. 1992; Fries et al. 1996b; Rath 1999), and can detect capped cells that are infested with mites, which are later uncapped and the mites removed or both the bee prepupae and the mites are buried together (Büchler et al. 1992; Fries et al. 1996b; Boot et al. 1997; Boecking and Spivak 1999).

18.6.2 *Euvarroa* Mites

A sister genus to *Varroa*, *Euvarroa* is a genus of parasitic mites that has been reported to colonize the Asian dwarf honeybees. *Euvarroa sinhai* Delfinado and Baker (1974) (the type species of the genus *Euvarroa*; Varroidae) was originally described from its host, *A. florea*, in India (Delfinado and Baker 1974). The biology of *Euvarroa* is similar to that of *Varroa*. The mites are only capable of reproducing in the drone brood cells of their hosts and disperse to other colonies via both drones and worker bees (Akratanakul and Burgett 1976; Mossadegh and Birjandi 1986; Aggarwal 1988; Kapil and Aggarwal 1989; Morin and Otis 1993). Similar to *Varroa*, *Euvarroa* causes little damage to their endemic host colonies. This may be because of the intensive grooming behaviour of the worker bees and the seasonal presence of drone brood cells that reduces the population of the mite to a minimal. However, evidence suggests that *E. sinhai* can reproduce in worker brood cells of *A. mellifera* (Mossadegh 1990). Also, Koeniger et al. (1993) reported that *E. sinhai* can survive in adult workers of *A. mellifera* and *A. cerana* in Thailand. However, the impact of the *Euvarroa* infestation in apiary colonies of *A. mellifera* or *A. cerana* has not been determined.

18.6.3 *Tropilaelaps* Mites

Twenty-five years ago, Burgett and Akratanakul (1985) predicted that in the near future *Tropilaelaps clareae* would play a major role in the destruction of commercial honeybee colonies far greater than that caused by *Acarapis woodi* and *Varroa* mites. Since then, *T. clareae* has been reported in countries beyond its primary host distribution (Kumar et al. 1993; Matheson 1996; Sammataro et al. 2000; Otis and Kralj 2001), but still no account of its presence in Europe and the Americas has

been reported. Because of the absence of *T. clareae* in western countries and the great interest in *Varroa* mites, the importance of *Tropilaelaps* mites has not been sufficiently recognized by bee researchers. Nevertheless, after the introduction of *A. mellifera* into Asia, cross infection of *T. clareae* from its original Asian honeybee hosts occurred resulting in a significant loss to commercial honey production (De Jong et al. 1982; Burgett et al. 1983; Bailey and Ball 1991). Burgett and Akwatanakul were not far off their prediction, particularly when one considers Asian apicultural industries.

18.6.3.1 Identity of *Tropilaelaps clareae* and Its Host Ranges

Tropilaelaps mites (Family Laelapidae) are obligate ectoparasites that feed on the haemolymph of larval honeybees. Both sexes of the mites are elongated and their bodies are covered with numerous, short, spine-like setae. *Tropilaelaps* are reddish brown in colour, though the males are less sclerotized. Compared to *Varroa* mites, *Tropilaelaps* are smaller, flatter and more oval shaped than round. The type species of the genus *Tropilaelaps* is *T. clareae* Delfinado and Baker (1961). *T. clareae* was first described from *A. mellifera* colonies in the Philippines and from rats living near bee colonies (Delfinado and Baker 1961). Its primary host species was later revealed to be *A. dorsata* from the Philippines (Bharadwaj 1968; Laigo and Morse 1968). Although current molecular evidence suggests that *A. dorsata*, host of *T. clareae* in the Philippines, is a distinct species but closely related to *A. breviligula* (Lo et al. 2010; cf. Chap. 1). *T. clareae* has been reported throughout most of the *A. dorsata* distribution in Asia (Matheson 1996; Anderson and Morgan 2007). In 1982, Delfinado-Baker and Baker described a second *Tropilaelaps* species, *T. koenigerum*, from *A. dorsata* colonies in Sri Lanka. Later on, *T. koenigerum* was reported in colonies of *A. dorsata* in India, Thailand and Borneo (Delfinado-Baker and Baker 1982; Koeniger et al. 2002; Tangjingjai et al. 2003) and in colonies of *A. laboriosa* in Nepal (Delfinado-Baker et al. 1985), which lives sympatrically with *T. clareae* in the same colonies. There are also reports on the occurrence of *T. clareae* on *A. cerana* and *A. florea* in Asia, though the impact on these honeybees is not severe (Delfinado-Baker 1982; Aggarwal 1988; Sihag 1988; Delfinado-Baker et al. 1989; Abrol and Putatunda 1995; Woyke 2005; Anderson and Morgan 2007). Before 2007, it was assumed that the mite that had become established in *A. mellifera* colonies in South Asia and the Oriental region and spread into Iran, Afghanistan, Kenya, South Korea and the Western Pacific Island of New Guinea was *T. clareae* (Burgett et al. 1983; Woyke 1984; Matheson 1996; Delfinado-Baker and Aggarwal 1987b; Kumar et al. 1993; Anderson 1994; Sammataro et al. 2000; Otis and Kralj 2001). However, Anderson and Morgan (2007), by examining the genetic variations, morphological variations and host associations of *Tropilaelaps* mites and their endemic hosts in Asia, showed that the once well-recognized '*T. clareae*' that infected *A. dorsata* and *A. mellifera* colonies throughout Asia and beyond was in fact comprised of two distinct species—*T. mercedesae*, which colonized *A. dorsata* and the introduced *A. mellifera* being one of the two.

18.6.3.2 *Tropilaelaps* Life Cycle

The life cycle of *Tropilaelaps* is similar to that of *Varroa*, except that *Tropilaelaps* can invade both worker and drone brood cells (Kapil and Aggarwal 1987, 1989), which can increase the mite population in a bee colony drastically compared to *Varroa* (Sammataro et al. 2000). The following description of the life cycle of *Tropilaelaps* is summarized from previous literature (e.g. Sammataro et al. 2000; Oldroyd and Wongsiri 2006) under circumstances where *T. clareae* (according to D. Anderson in Oldroyd and Wongsiri 2006) infected an *A. mellifera* colony. Studies of the life cycles of different *Tropilaelaps* species on their native hosts are yet to be investigated. In an established bee colony, a gravid female mite enters a brood cell before capping, feeds on larval haemolymph for about 2 days or less and then lays her first egg. Three or four eggs can be found in a brood cell per reproductive cycle.

Normally, the first egg will develop into an adult male. The developmental time from egg to adult takes about 6–7 days. The mother mite and her offspring will emerge from the brood with the adult bee and can enter other brood cells directly (for the mother mite) or mate with the opposite sex in the bee colony (for the virgin offspring). In a period when the host queen bee does not lay eggs, adult *Tropilaelaps* will adopt a phoretic stage and stay on the bees' sclerites until there are brood cells to parasitize, though no more than 3 days (Koeniger and Muzaffar 1988; Rinderer et al. 1994; Wilde 2000). This might explain the mechanism by which *A. dorsata* and its related giant honeybee species have a reduced load of *Tropilaelaps* through seasonal migration (Kavinseksan et al. 2003).

18.7 Tracheal Mites of the Genus *Acarapis*

Acarapis woodi (Prostigmata; Tarsonemidae) invades the tracheal system of adult honeybees and feeds on the haemolymph by piercing the tracheal wall (Hirschfelder and Sachs 1952). In North America, colonies that are infested with *A. woodi* suffer symptoms such as brood decline, decrease in worker bees and low honey production (Royce and Rossignal 1989; Morse and Nowogrodzki 1990; Bailey and Ball 1991; Mussen 2001). In serious cases, an *A. woodi* population can overwhelm and kill the entire colony. In *Apis mellifera*, the entire life cycle of *A. woodi* from egg to adult can be completed in about 2 weeks with males maturing a couple of days earlier than females, which contributes to the explosion of *A. woodi* population in a colony in a short period of time (Pettis and Wilson 1996; Wilson et al. 1997). All life stages of *A. woodi* are spent living inside the tracheal system of the bees, except for the adult females that sometimes venture out to find a new host (Sammataro and Needham 1996). Most dispersing female mites are attracted to the prothoracic spiracle of the adult bees and will eventually reside and lay their eggs in the trachea (Hirschfelder and Sachs 1952; Phelan et al. 1991). Adult female mites can survive outside of the host for only a few hours (Hirschfelder and Sachs 1952). Since the discovery of *A. woodi* in the Isle of Wight, England, during the early twentieth century

(Clark 1985; Sammataro 1995), there have been few reports on the occurrence of *A. woodi* in Asian honeybees. Until now, there are reports from the subcontinent of India–Pakistan where *A. woodi* was found infesting *Apis cerana* and *A. dorsata* (Dhaliwal and Sharma 1974; Adlakha 1976; Delfinado-Baker et al. 1989; Abrol 2000). Accounts on the demise of managed *A. mellifera* colonies in Asia as a result of *A. woodi* have never been reported.

However, because of the small size of the mites which cannot be observed with the naked eye, beekeepers cannot detect the mites at the initial stage of a mite infestation. When the colony progressively deteriorates, beekeepers notice the stress signs of the colonies such as declining populations and weak worker bees with deformed wings and metasoma (Sammataro 1995). These might be attributed to other causes that are more visibly conspicuous such as endemic *Varroa* or *Tropilaelaps* mites. Methods for the diagnosis of tracheal mites are also tedious, time consuming, and require experience to perform (Ragsdale and Furgala 1987; Fichter 1988; Ragsdale and Kjer 1989; Shimanuki and Knox 1991; Grant et al. 1993). Hence, reports of *A. woodi* in Asia might have been overlooked because the maladies can superficially resemble symptoms that are caused by other agents.

18.8 Non-parasitic Mites Associated with Asian Honeybees

Parasitic mites described in the earlier sections of this chapter represent only a minor fraction of the diversity of Acari associations with honeybees. Most Acari found in the nests of honeybees usually have a saprophagous lifestyle (Eickwort 1990) and feed on fungus-infected debris in the hives, dead bees and sometimes pollen (kleptophages). Three main orders of non-parasitic mites are commonly found in honeybee colonies: Astigmata, Prostigmata and Mesostigmata. Mites of the order Astigmata, currently placed in the order Oribatida (Krantz and Walter 2009), are the most abundant in the colonies of honeybees (Eickwort 1990). Two subfamilies of the family Acaridae, particularly Forcelliniinae and Horstiinae, are frequently found (O'Connor 1982, 1988; Delfinado-Baker and Baker 1987). Although primarily a myrmecophilous species, *Forcellinia faini* (Forcelliniinae), can be found on the hive floors of *A. cerana* colonies in Thailand (Fain and Gerson 1990). Among the prostigmatid mites, except for the parasitic tarsonemid mite on honeybee, *A. woodi*, members of this family are fungivorous and occur facultatively in honeybee colonies (Lindquist 1986). Generally, prostigmatid mites associated with social insects are known to have relatively short life cycles and are commonly phoretic (Lindquist 1986; Eickwort 1990). The fore-tarsal claws of these mites are usually enlarged to hold onto the setae of their hosts. Sumangala and Haq (2002) reported that *Pseudoacarapis indoapis* (Tarsonemidae) is found in *A. cerana* colonies in India feeding on fungal debris and stored pollen. Mites in the order Mesostigmata are primarily free-living predators, but many lineages have evolved parasitic lifestyles with other arthropods, e.g. *Varroa* mites (Hunter and Rosario 1988). Many mesostigmatid mites found in honeybees are free living and feed on other saprophagous mites, insects, fungi

and pollen. *Neocyphophylax indica* (Family Ameroseiidae) is a common facultative kleptophage of *A. cerana*, *A. florea* and *A. dorsata* in Asia (Delfinado-Baker et al. 1989; Needham et al. 2001). The primary habitat of *N. indica* is on subtropical and tropical tree flowers where it feeds on pollen; however, during a foraging trip of a worker bee, this mite can ‘hitch-hike’ on the mesosoma or metasoma to be brought back to the colonies, where the mite then feeds on stored pollen (Delfinado-Baker et al. 1989; Haq et al. 2001). Another Ameroseiidae species is also reported to be found associated with *A. cerana*, i.e. genus *Afrocyphophylax* (Delfinado-Baker et al. 1989). Works on the diversity and life history of the non-parasitic Asian honeybee mites are progressing more slowly than the corresponding parasitic groups. This may result from the non-commercial importance of the non-parasitic mites so that they receive less attention from bee researchers. Further investigations into population diversity of Asian honeybees will undoubtedly result in the discovery of additional unknown non-parasitic mites associated with honeybees in Asia.

Although we have information on life history and biology of honeybee mites, little information is available regarding variations in the genetics, behaviour and pathology of mites from different locations. Future genetic and experimental work focusing on the population level of the mites can be of immense importance to the discovery of new genetic types of mites and their corresponding levels of pathogenicity on their native host populations and on *A. mellifera*. Besides, studies of the Asian honeybee populations can reveal significant information regarding where to probe for genetically distinct types of mite populations. Microbes, particularly viruses associated with the mites, are also another subject of interest that will expand our knowledge for understanding the interactions among the hosts, parasites and pathogens. Biogeographical studies of the patterns of co-evolution between the honeybee and mite populations could well benefit from including the pathogen perspective to elucidate a clearer understanding of these complex honeybee–mite associations in Asia.

18.9 Management Strategies of Honeybee Diseases

A number of strategies have tentatively been applied in recent years to protect honeybees against pathogens and parasites. These include a broad range of chemotherapeutic compounds that have been tested for their ability to control honeybee diseases. Most of these products were promising in terms of controlling pathogen growth either in culture or in bee colonies. Unfortunately none of the tested compounds achieved a complete control of the diseases that could irradiate them (Lodesani and Costa 2005).

Facing these challenges of partially active molecules, a series of alternative strategies has been developed and implemented to control honeybee diseases. These methods include the use of disease-resistant bee lines that are currently being deployed by many breeding programs. In recent years, the recourse to biological control agents has been gaining ground and seems to be promising. This relies on the exploration of beneficial microorganisms that antagonize with honeybee pathogens and/or the eco-friendly natural products.

18.9.1 Antibiotics and Fungicide Molecules

One of the earliest strategies that have been implemented to control pathogens and parasites threatening honeybees was the use of antibiotic and fungicide molecules. Over the course of its development, this strategy faced numerous limitations including the low number of available molecules, their lack of specificity in terms of action and the quick development of resistance.

The first pesticides released for use by beekeepers included pyrethroids and organophosphates (Milani and Barbattini 1988). These molecules were used to control hive parasites such as mites. Their use has substantially increased the cost of production whilst never provided a complete control of the parasites. In addition, mites developed resistance over time reducing more and more the efficacy of the used molecules. At present, Apistan, a product containing the synthetic pyrethroid fluvalinate, completely lost its effectiveness for the control of *V. destructor* due to the parasite evolution of resistance. It has been replaced with plastic strips containing the organophosphate coumaphos, the efficacy of the latter did not last long (Trouiller 1998; Elzen et al. 1999) and has currently been substituted by Amitraz, a triazapentadiene compound of an unknown action. To guarantee efficacy, beekeepers tend to increase doses and rates and to use various product mixtures. Some chemicals, particularly fluvalinate, may accumulate in wax, exposing honeybees to levels of chemical residues that are harmful to worker bees longevity (Chauzat et al. 2009; Johnson et al. 2009) and may pollute the honey and other hive products (Martel et al. 2006).

Tetracycline antibiotics, such as oxytetracycline hydrochloride (OTC), have been used to control beehive diseases such as AFB. In recent years, tetracycline-resistant strains have been emerging in many countries including USA, Canada and Argentina (Cox 2000; Miyagi et al. 2000; Alippi et al. 2007). Treatment of hives with oxytetracycline hydrochloride may mask disease signs for several months while *P. larvae* spores may be still found in the honey. The OTCs were also used against *M. plutonius* infection. The efficacy of other antibiotics, such as tylosin and lincomycin and their derivatives have also been tested against AFB (Fedlaufer et al. 2001; Kochansky et al. 2001; Elzen et al. 2002; Alippi et al. 2005; Pettis and Feldlaufer 2005). Among these, tilmicosin is a semi-synthetic macrolide antibiotic synthesized by a chemical modification of tylosin and exclusively developed for veterinary use (Reynaldi et al. 2008). Although there is a lack of information regarding its use, it has been approved to control causal agents of respiratory diseases in farm animals, including gram-positive bacteria, mycoplasma and some gram-negative bacteria (Shryock et al. 2002). Likewise, the fumagillin fungicide was used against *Nosema apis* and was shown to suppress infection when applied at doses ranging from 0.005 to 0.03 mg mL⁻¹, without detectable side effects.

18.9.2 Genetic Breeding

In the early 1990s several breeding programs were implemented to develop honeybee population with resistance/tolerance to major diseases and/or with the hygienic

behaviour (Spivak and Gilliam 1998). The hygienic behaviour consists of selecting and/or training bred bees to early detect and discard diseased larvae before they become infectious towards the whole colony. Hygienic behaviour in bees is defined as the ability of bees to detect and remove diseased or parasitized brood. It is considered the primary mechanism of honeybee resistance to a variety of brood diseases (Spivak and Gilliam 1993; Spivak 1996). Hygienic bees have an acute sense of smell and are able to detect affected cells very soon after infection and remove them before large numbers of spores have been produced (Spivak et al. 2003). The importance of this behaviour in breeding comes from the fact that bees are able to detect infected brood better than any beekeeper specialist, hence preventing the spread of the disease to the healthy member bees in the colony.

The genetic analysis of the hygienic behaviour conducted in the early 1960s (Rothenbuhler 1964) and in recent years (Spivak and Reuter 2001; Lapidge et al. 2002) revealed that this trait is recessive and under a complex genetic control and involves a number of genes whose products interact in a complex way, and demonstrated that increased genetic diversity in bees may have an important function in reducing the likelihood of outbreaks of the disease (Tarpy 2003; Evans and Spivak 2010).

18.9.3 Sanitation Practices

Management and sanitation strategies are directed towards helping bees defend against infection or avoiding infection in the first place. These practices include supplemental feeding to improve the nutritional and health status of bees, keeping hives clean and well ventilated (Gochnauer et al. 1975), replacing storage and brood combs annually and avoiding transfer of combs between colonies (Malonova and Titera 1995; Flores et al. 2005). Several different sterilization methods have been tested in attempts to reduce spores load in beehives. Fumigation of beehive equipment using various chemicals was performed by Gochnauer and Margetts (1980) and Faucon et al. (1982), but this was not widely accepted due to residuals found in both wood and wax. Some beekeepers have also tried fumigation with lactic, formic and oxalic acid (Calderone 1999; Higes et al. 1999; Dodologlu and Emsen 2009) to combat the mite. Although these approaches do not replace insecticides in the colonies, they are somewhat less effective in controlling mites and can directly be toxic to the bees. Lactic acid (15 %, v/v) sprayed on combs covered with bees results in 90 % decrease in mite population (Rendall 1996), while formic acid has been used successfully in Germany by soaking an absorbent pad held in a cradle over the brood nest (Rendall 1996). Formic acid is also used on soaked pads in Italy with an efficacy of up to 98.8 %, when used every third day for three weeks (Mutinelli et al. 1994).

Gamma irradiation from a cobalt-60 source was effectively used to sterilize contaminated beekeeping equipment (Hornitzky and Willis 1983). Irradiation was also tested to sterilize wax and honey (Wooton et al. 1985). At the optimum level of radiation there were no negative effects detected on wax composition though some

physico-chemical alterations were observed in honey, including decreases in enzymatic activities and a change in colour (Baggio et al. 2005). However, the accessibility of radiation facilities is the limiting factor of this technology. Likewise, sterilization of honey using heat, although efficient, shows several limitations. Current research efforts are focused on other alternative methods such as microwave radiation, infrared heating, ultrasonication and ultrafiltration to preserve honey quality (Subramanian et al. 2007).

18.9.4 Biological Control Agents

Considering the worldwide spread of honeybee diseases and the lack of registered and effective molecules to fight them, there is a great interest in developing alternative control methods. Non-contaminating natural biocides produced by biological control agents (BCAs), although still a great challenge, will help manage bee disease and improve the quality of honey and other by-products while avoiding the presence of undesirable residues.

18.9.5 Antagonistic Bacteria and Fungi

A broad range of bacteria have been tested on *Paenibacillus larvae* in culture, most of which were antagonistic and exhibited an antibacterial activity against pathogen found in the gut of *A. mellifera*. Most of these bacteria belonged to *Bacillus* species (Evans and Armstrong 2005, 2006). The bacterial communities occurring in the digestive tract of the Japanese honeybee, *Apis cerana japonica*, were also assessed and investigated by in vitro inhibition assays and their ability to inhibit *P. larvae* was determined (Yoshiyama and Kimura 2009). Alippi and Reynaldi (2006) investigated the potential use of aerobic spore-forming bacteria isolated from honey samples and other apiarian sources as biocontrol agents against *P. larvae*. Interestingly, species of *Bacillus*, *Paenibacillus* and *Brevibacillus* frequently isolated from apiarian sources (Gilliam and Prest 1978) have been reported to be effective biocontrols by producing antibiotics and antifungal metabolites (Nielsen and Sorensen 1997). Most of the isolates exhibiting very strong activity against the honeybee pathogens were identified as *Bacillus* spp. (*B. megaterium*, *B. licheniformis* and *B. cereus*). Furthermore, a new bacterial isolate identified as *Paenibacillus polymyxa* showed a high level of antimicrobial activity against *P. larvae* (Lee et al. 2009). Recently, a new study demonstrated the presence of actinomycetes associated with hives of bees from the genus *Apis* and *Trigona*. These actinomycetes were able to produce antibacterial activity against bee pathogens *P. larvae* and *M. plutonius*, which provides a new source of microorganisms able to produce novel antibiotics to combat bee diseases in the beekeeping industry (Promnuan et al. 2009). Likewise, many other microbes associated with honeybees, such as certain *Penicillium* and *Aspergillus*, showed inhibiting effects on growth of *Ascospaera apis* in culture (Gilliam et al. 1988b).

18.9.6 *Natural Products*

Many natural compounds have been tested in honeybee colonies and on the pathogens in culture in an attempt to control chalkbrood and AFB (Heath 1982). Some of them include natural plant-derived antimicrobial/antifungal compounds (Aronstein and Hayes 2004; Mourad et al. 2005; Gende et al. 2008). Essential oils containing citral and geraniol were reported to have the best inhibiting effect on fungal growth in vitro (Calderone et al. 1994). Bailac et al. (2006) showed that oils whose composition has mainly benzenic compounds such as cinnamon oil (*Cinnamomum zeylanicum*) presented a good antimicrobial activity against strains of *Paenibacillus larvae* subsp. *larvae*.

Propolis, another natural product derived from plant resins and produced by honeybees to seal the walls and entrance of the hive, contributes to the protection of the colony against different pathogens and has antimicrobial properties (Ghisalberti 1979). Propolis has empirically been used in apiculture for the prevention of AFB and other honeybee diseases for years (Mlagan and Sulimanovic 1982) and it is well known for its strong inhibitory effects against gram-positive and gram-negative bacteria in laboratory cultures (Drago et al. 2000; Sforzin et al. 2000; Bastos et al. 2008).

The antibacterial activity of propolis could be related to the chemical composition of propolis, which includes phenolic compounds (flavonoids and aromatic acids) (Arfaoui et al. 2007), terpenes and essential oils among others (Marcucci 1995; Kumazawa et al. 2002). Phenolics are well known as antimicrobial compounds with antibacterial and antifungal activity. The in vitro challenge of many fungi with these compounds revealed their effectiveness in reducing fungal growth (Arfaoui et al. 2006).

18.9.7 *Mechanisms of Action of Biological Control Agents*

If biological control is to become a viable alternative for honeybees' protection, a large number of BCAs with various modes of action must be isolated and identified. Recent studies showed the deployment of several BCAs with effective methods for the selection of antagonistic microorganisms as well as the establishment of in vitro and in vivo disease challenge experiments using adult bees and larvae (Flores et al. 2004; Bastos et al. 2008). Multiple modes of action were determined. These include the production of antibiotics, antibiotic-like compounds, bacteriocins and antifungal metabolites, (Alippi and Reynaldi 2006), the stimulation of bee's immune system (Evans and Lopez 2004) and the enhancement of the defence response of honeybees (Antunez et al. 2008). Other factor may be implicated in the interaction: Honeybee–Biological control agent–Pathogen.

18.9.7.1 Antibiosis

Many bacterial species have been shown to inhibit the growth of bee's pathogens in vitro as well as to reduce disease symptoms in vivo through the production of secondary metabolites which are used in direct antagonism with pathogens and pests (Alippi and Reynaldi 2006; Lee et al. 2009; Yoshiyama and Kimura 2009). The ability to produce multiple classes of antibiotics that differentially inhibit bee pathogens is likely to enhance biological control. Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens. For example, many *Bacillus* species inhibiting the growth of *P. larvae* and *A. apis* by producing a number of antibiotics were identified (Nielsen and Sorensen 1997). These species are known for their high production of a variety of secondary metabolites such as antibiotics, bioinsecticides, enzymes and lipopeptides (Phister et al. 2004). Sabate et al. (2009) were able to isolate three different *B. subtilis* strains that can inhibit two important honeybee pathogens by two different mechanisms; that is surfactin synthesis and fungicide or cell-to-cell interaction.

More recently, a *Paenibacillus polymyxa* with a high antibacterial activity against *P. larvae* was isolated from honey (Lee et al. 2009). This species has been known to produce polymyxins with broad spectra of activity that includes gram-negative and gram-positive pathogenic bacteria (Storm et al. 1977). Polymyxins are classified as peptide antibiotics synthesized by multi-enzyme complexes. More than 15 polymyxins have been identified which are known to bind to lipid A of the bacterial cell membrane, resulting in membrane disruption and cell death (Martin et al. 2003). Actinomycetes, known for their secondary metabolites, which have been successfully used as drugs in human and veterinary medicines, have been shown to inhibit the growth of *P. larvae* in vitro by producing antimicrobial compounds (Promnuan et al. 2009) yet to be identified. Out of more than 43,000 known bioactive natural products almost one fourth are produced by actinomycetes (Lazzarini et al. 2000). The most important genus is *Streptomyces* with over 500 described species producing many important antibiotics, including streptomycin. Other antibiotics produced by *Streptomyces* sp. include spectinomycin, neomycin, tetracyclines, nystatin, erythromycin and chloramphenicol.

18.9.7.2 Stimulation of the Bee Immune System

Besides their group strategies to combat disease such as grooming, nest hygiene and other behavioural traits which can reduce the impacts of many parasites and pathogens (Spivak and Reuter 2001), honeybees also possess individual defence mechanisms, including immune responses towards pathogens (Evans 2004). These involve diverse set of actions including the secretion of antimicrobial peptides, phagocytosis, melanization and the enzymatic degradation of pathogens (Hoffmann 2003).

Most research on honeybee immune traits has focused on responses towards bacterial threats, towards which both larval and adult bees are vulnerable (Casteels et al. 1990; Evans et al. 2006). Evans and Lopez (2004) showed that non-pathogenic

bacteria can stimulate the immune response of honeybee larvae, helping bees survive exposure to pathogens. They also found that non-pathogenic bacteria can generate a sustained increase in levels of the antibacterial peptides abaecin and defensin. The authors proposed then that the mixture of non-pathogenic bacteria could be presented as a potential surrogate for assaying the immune responses of different honeybee lineages and that non-pathogenic bacteria can be used as a probiotic to enhance honeybee immunity, helping bee larvae and other life stages survive attacks from pathogens in the field.

Antunez et al. (2008) proposed a possible indirect effect of the propolis such as the stimulation of the bee immune system as the same product was able to enhance innate and adaptive immune responses of mouse, bovines and humans. In vitro and in vivo assays demonstrated that propolis activates macrophages, increasing their microbiocidal activity, enhances the lytic activity of natural killer cells and stimulates antibody production (Sforzin 2007).

18.10 Conclusion

With the increasing demand on organic honey and the reduction of increased dependence on synthetic pesticides and antimicrobial compounds that often lead to a general deterioration of honeybee colonies and the environment, it is quite obvious that an integrated pest management (IPM) approach is needed to guarantee the sustainability of the beekeeping industry. Several steps are required for the settlement of such a successful approach, including an appropriate management and sanitation of the hives and the use of honeybee disease-resistant lineage. The ultimate step will be to deploy biocontrol agents, introduced to honeybees through various method of application such as spraying, feeding, evaporating, or fumigating, to reduce disease levels and prevent the development and spread of pathogens. Recent findings gathered using biocontrol agents, such as propolis, essential oils, probiotic bacteria and gut microbial communities, seem promising and it would be conceivable to combine them within an IPM strategy to manage honeybee pathogens and pests. Nevertheless, more research is still needed to examine the mechanisms governing the action of biocontrol agents and guarantee their safe deployment.

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Chapter 19

Impact of Climate Changes

19.1 Introduction

Bees of the *Apis* genus are distributed throughout the world in highly diverse climates. The *Apis mellifera* species, whose distribution range extends to sub-Saharan Africa, northern Europe, and Central Asia, is found in a wide variety of environments, including the oases of the African desert, the Alps, the fringes of the tundra, and the mists of the UK. Its ecotypes have adapted remarkably well to their biotopes. The other honeybee species of the *Apis* genus are distributed around Asia, particularly tropical Southeast Asia (Ruttner 1988).

The honeybee is a eusocial insect whose role in plant pollination is essential to global ecology (Poinar and Danforth 2006). The discovery of a 100-million-year-old amber fossil pushes back the date when bees began pollinating flowers by millions of years (The Honeybee Genome Sequencing Consortium 2006), although species of *Apis* are much younger than this. Honeybees defend their nest, brood, and stored food through many complicated and efficient defensive behaviors (Breed et al. 2004), but populations have decreased rapidly in many countries in recent years. Explanations for this decline include global warming caused by the greenhouse effect, indiscriminate use of pesticides, reduction or extinction of plant species, and other human-induced disturbances. In addition, infection by strong pathogenic microorganisms (such as viruses) or pests (such as mites) have been cited as important direct or indirect reasons for honeybee collapse (Chen and Evans 2007; Chen et al. 2008; Higes et al. 2006; Klee et al. 2006; Santillán-Galicia et al. 2008; Yang 2005). Protection of the honeybee species is a worldwide concern.

The Asiatic honeybee, *A. cerana* Fabricius, plays an important role in maintaining the biodiversity of plants in Asia. *A. cerana* is still found in the wild, where it nests in tree holes, fallen logs, and crevices, but it is also one of the few bee species that can be domesticated. It has acquired some incomparable advantages in its long history of evolution compared with other introduced *Apis* species. It has adapted to adverse climatic conditions, and can survive extreme fluctuations in temperature and long periods of rainfall. For example, it can survive when the air temperature is as low as -0.1°C , a temperature lethal for the Western honeybee. It is a natural host for, and can tolerate the mite, *Varroa destructor*, and the microsporidian, *Nosema ceranae*,

both of which are the serious pests of the Western honeybee (Kasprzak and Topolska 2007; Williams et al. 2008). Having coevolved with these parasites, the Asiatic honeybee exhibits more careful grooming behavior than the Western honeybee, and appears to have other more effective defenses against these parasites. The Asiatic honeybee can also effectively pollinate mountain plants and crops, especially the early flowering fruits and vegetables in high altitude regions, such as the Himalayan region of China where temperatures are too low for the exotic *A. mellifera*. Nevertheless, over the last 20 years, it has become a threatened species in China for many reasons, most likely because of competition from the introduced Western honeybee, *A. mellifera*. More research is needed and urgent measures must be taken to protect the Asiatic honeybee. It is noteworthy that the Asiatic honeybee does not produce propolis, a highly valued resinous material that is used to seal small open spaces in the hive, and is believed to be involved in the honeybee defense system against pathogens in the hive.

Honeybees defend against many pathogens by producing antibiotic substances such as propolis and royal jelly (Souza et al. 2007; Fontana et al. 2004). The innate immune system is the first line of defense against pathogens in plants and invertebrate animals, and it is also critical for vertebrate immunity before the acquired immune system generates a specific response (Girardin et al. 2002; Loker et al. 2004; Muller et al. 2008). Various antimicrobial peptides are the key elements of the insect immune system (Boman 1995; Bulet et al. 1999; Hoffmann et al. 1999). After the honeybees are infected by pathogens, four antimicrobial peptide families are synthesized, representing a broad spectrum of antimicrobial activity in the hemolymph. All of these are cationic peptides identified as: apidaecins (Casteels et al. 1990), abaecin (Casteels et al. 1990), hymenoptaecin (Casteels et al. 1993), and defensin (Casteels-Josson et al. 1994). Recently, two structurally different defense genes were cloned from *A. mellifera* (Klaudiny et al. 2005). Almost all of the honeybee antimicrobial peptides and the antimicrobial peptide genes are reported from the Western honeybee; however, little research has been conducted on the Asiatic honeybee. As a very ancient and important native honeybee species in China, many aspects of the species need to be explored.

Xu et al. (2009) designed primers for the sequences of the four antimicrobial peptide cDNA gene families (abaecin, defensin, apidaecin, and hymenoptaecin) of the Western honeybee, *Apis mellifera* L. and identified all the antimicrobial peptide cDNA genes in the Asiatic honeybee for the first time. All the sequences were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR). In all, 29 different defensin cDNA genes coding 7 different defensin peptides, 11 different abaecin cDNA genes coding 2 different abaecin peptides, 13 different apidaecin cDNA genes coding 4 apidaecin peptides and 34 different hymenoptaecin cDNA genes coding 13 different hymenoptaecin peptides were cloned and identified from the Asiatic honeybee adult workers. Detailed comparison of these four antimicrobial peptide gene families with those of the Western honeybee revealed that there are many similarities in the quantity and amino acid components of peptides in the abaecin, defensin, and apidaecin families, while many more hymenoptaecin peptides are found in the Asiatic honeybee than in the Western honeybee (13 versus 1). The

results indicated that the Asiatic honeybee adult generated more variable antimicrobial peptides, especially hymenoptaecin peptides than the Western honeybee when stimulated by pathogens or injury. This suggests that compared to the Western honeybee that has a longer history of domestication, selection on the Asiatic honeybee has favored the generation of more variable antimicrobial peptides as protection against pathogens.

Climate change estimations predict upheavals in certain regions of the world a few decades from now, with encroaching deserts, a retreating icecap, snowmelt, changing rainfall patterns, and a greater frequency of extreme climate events generally. A change in climatic conditions is bound to have an impact on the survival of these ecotypes or of honeybee species that are closely associated with their environment. Migration and changes in their lifecycle and behavior could help them survive in new biotopes. As the honeybee's genetic variability is crucial to its adaptation, we would ensure that we preserve this genetic variability. Honeybees will also need to adapt to a whole array of predators, parasites, and pathogens surrounding them. Not only will the relationships between hosts and parasites change, but honeybees will also have to cope with new stresses arising from trade-facilitated transfers of pathogens among honeybee species. In such a context, climate change could create new opportunities for establishing honeybees in undreamt-of regions or habitats

19.2 Impact of Climate Change on Honeybees

Climate change can impact honeybees at different levels. It can have a direct influence on honeybee behavior and physiology. It can alter the quality of the floral environment and increase or reduce colony harvesting capacity and development. It can define new honeybee distribution ranges and gives rise to new competitive relationships among species and races, as well as among their parasites and pathogens. Beekeepers will also be obliged to change their apiculture methods. They will favor moving their hives to new foraging areas and importing foreign races to test their value in the new environments.

19.3 Impact of Climate Change on Honeybee Behavior

The European honeybee, *A. mellifera*, has the potential to adapt to hot climates. For instance, *Apis mellifera sahariensis* is found in the oases of the Sahara, where it has adapted to local bloom (such as palm flowers) and extreme heat (Ruttner 1988). In the USA, honeybees can develop in the Arizona Desert. The survival requirement for these bees is a supply of water, which they use in large quantities to raise their larvae and to regulate the brood temperature between 34 and 35 °C. In an arid environment, desert flowers are unable to provide the bees with enough water and they die. According to climate change predictions, desert regions will

become even drier, leading to the disappearance of oases and their honeybees. *A. mellifera sahariensis* is highly unlikely to migrate naturally to more favorable desert areas because oases are very isolated and not conducive to long-distance migration or swarming. It is therefore vital to envisage conservation measures to transfer this bee to zones favorable to its development, lest we lose this ecotype that is so valuable for world biodiversity.

Climate change can influence the honeybee development cycle. It is generally agreed that each race of honeybees develops at its own rate (Louveaux et al. 1966). Any sort of climate change or movement of a race of honeybees from one geographical region to an alien one is therefore bound to have measurable consequences. In cool regions, honeybees spend the winter clustered in a tight ball and use their honey stores to provide them with the energy they need to survive until spring. The honeybee's capacity to accumulate energy reserves and to manage the colony's development exerts significant adaptive pressure. In the spring, when the weather becomes more clement, the queen starts to lay eggs and the colony develops and increases the size of the worker population. A cold snap lasting several weeks may occur during which the honeybees are unable to harvest. The large size of the honeybee population causes such a rapid depletion of stores that the colony can die of starvation. It is something that can easily happen to hybrid bees (crosses of several races by bee breeders), which develop very fast in spring. In contrast, local ecotypes that are better adapted to the environmental conditions are more cautious and develop more slowly in spring until after this cold snap, when they breed very rapidly. In this way they avoid jeopardizing the colony's survival. A distinction therefore needs to be made between local ecotypes, which need to adjust their development and stores to the climate, and hybrid bees selected by bee breeders. Hybrids have not been bred to build up food stores, the queen does not adjust her egg-laying, and the workers do not adjust their larvae-rearing, with the result that the bees are unable to survive without the assistance of a beekeeper to provide them with unlimited supplies of sugar solution (Louveaux et al. 1966). The variability of the honeybee's life history traits with respect to temperature and the environment shows such plasticity and genetic variability that this could give rise to the selection of development cycles suited to new climatic conditions. Bees adjust their behavior to weather conditions. They do not go out when it rains and, in extremely hot weather, they gather water to keep the colony cool.

19.4 Climate-Induced Changes in Flora and Honeybees

Climate influences flower development and nectar and pollen production, which are directly linked with colonies' foraging activity and development (Winston 1987). Bees must build up sufficient honey stores to enable them to survive the winter. The nurse worker bees must consume enough pollen to feed the larvae through their pharyngeal glands. A major effect of climate change on honeybees stems from changes in the distribution of the flower species (Thuiller et al. 2005) on which the bees

depend for food. Will plants be able to survive the rapid advent of drought conditions or, on the contrary, wetter seasons? If they can, will conditions be optimal for flowers to produce the nectar and pollen needed for honeybees to develop? Although we do not know the precise impact that these factors could have on honeybees in a context of climate change, there is a large body of data at our disposal indicating that environmental changes have a direct influence on honeybee development. We are aware of the impact that rain can have on honey harvesting by bees. For instance, when acacia flowers are washed by rain, they are no longer attractive to honeybees as it dilutes their nectar too much. Likewise, an overly dry climate will reduce the production of flower nectar for honeybees to harvest: lavender flowers produce no nectar when the weather is too dry, which makes harvesting by bees a largely hypothetical matter. In extreme situations, honeybees can die of starvation unless the beekeeper is vigilant. The honeydew produced by stinging insects from certain plant species is also climate-dependent. In Alsace, very special conditions are required for the development and growth of balsam fir aphid populations, whose honeydew is highly attractive to honeybees (Jean-Prost and Le Conte 2005). On the other hand, certain types of honeydew cause dysentery in honeybees.

19.5 Climate Change and Honeydew Production

The food shortages stemming from an excessively dry climate, which reduces pollen production and impoverishes its nutritional quality, are currently the subject of much debate (Stokstad 2007). Honeybees that are born in autumn spend the entire winter in the hive and form the backbone of the colony in spring. A pollen diet is very important for rearing the future workers (Mattila and Otis 2006). A pollen shortage induced by autumn drought will have the effect of depriving bees in winter, weakening their immune system and making them more susceptible to pathogens, and shortening their life span.

Tropical climates may evolve towards more distinct seasons with dry periods. In this case, Asian honeybees would need to rapidly step up their honey-harvesting strategy to amass sufficient stores to survive periods without flowers. As an alternative, they could develop a migration strategy, as has *Apis dorsata*, a giant honeybee of the *Apis* genus. *A. dorsata* colonies build their nests in the open air, consisting of a very big, single comb of up to 2 m in length. *A. dorsata* tend to be gregarious, which gives them a distinct advantage in the joint defense of their nests against predators. *A. dorsata* honeybees readily migrate in response to seasons, flowering patterns, or disruption. They abandon their nests and can fly distances of up to 200 km to escape starvation or predators. After leaving their nests unoccupied for several months (or in some cases 1 or 2 years), the same honeybee colony returns to colonize the same nest in the same tree every year (Neumann et al. 2000; Paar et al. 2000). A plausible scenario is that, to guarantee their survival, these honeybees will migrate in step with the evolution and disruptions of local flower species, and that they will switch their migration sites, abandoning regions that have become too arid. What will happen to

honeybee species that do not migrate? If there is large-scale natural swarming, these species will be able to swarm to more favorable regions and abandon their regions of origin. Failing that, they will need to evolve rapidly towards a harvesting strategy to enable them to survive during periods with no flowers. A commonly cited textbook example is that of the Landes ecotype in south-western France. In the Landes region, the colonies develop in step with heather bloom, which is the main natural resource for these honeybees. The Landes ecotype has therefore modeled its development on that of the plant (Louveau 1973). A change in climate is bound to alter the flora. What will this do to the heather and to this honeybee ecotype?

19.6 Impact of Climate Change on Geographical Distribution of *A. mellifera* and Other Races

19.6.1 Natural Movements

As with other arthropods (Chown et al. 2007), climate change will lead to a reduction or an increase in the areas available to honeybees. Bees will abandon areas that evolve towards drought and migrate towards the fringes of such areas. In contrast, honeybees will colonize cold areas that were initially hostile to them. A well-studied example is that of the Africanized honeybee. The Africanized honeybee's geographical distribution has now extended as far as Argentina and the USA, where it has come to a halt (Diniz et al. 2003; Pinto et al. 2005). According to researchers, this is because the climatic conditions beyond that are too cold for the Africanized honeybee. Global warming is therefore conducive to the bee's expansion outside its current distribution range. Moreover, the Africanized honeybee is less susceptible to the *Varroa* mite than European honeybees (Martin and Medina 2004). It is therefore expected to form feral colonies and to adapt more easily than other races, which is what is happening in the USA at present.

19.6.2 Movements by Beekeepers

Beekeepers are expected to change their transhumance habits and abandon areas that have become too dry in favor of wetter areas. They will most probably be tempted to continue importing queens of other races to test their potential to adapt to new climates. Although such imports will increase the genetic diversity of honeybee populations, they will also act as vectors for the introduction of new pathogens or of new bee haplotypes of varying usefulness, as discussed earlier (see the part on Africanized honeybee).

Bringing imported honeybees into contact with local races and ecotypes facilitates a genetic admixture that may aid the survival of the species but will also tend to eradicate local ecotypes and pure races through genetic pollution.

19.7 Adaption Potential

A. mellifera is a species that has shown great adaptive capacity, as it is found almost everywhere in the world and in highly diverse climates. Imported to the Americas by the colonists, it has coevolved with humans and has spread throughout the continent, from north to south. It may be assumed that, as the species has great biodiversity, it will be able to use its genetic variability (Cornuet and Louveau 1981) to adapt to climate change. In contrast, the Asian species have remained in Asia, which might indicate lesser adaptability to different environments and fragility in the face of climate change. *A. mellifera* seems to have more adaptive potential than its Asian cousins, which have low yields and have been subject to little transhumance. Humans, with whom *A. mellifera* has coevolved for several centuries, will certainly be decisive in helping honeybees to survive in hostile environments and in preserving the biodiversity of these species. Beekeeping is an essential pollination and production support tool in this respect. However, if bee ecotypes are no longer suited to their biotopes, feral colonies will need to evolve rapidly to survive without human assistance.

19.8 Trade in Honeybees Affecting Diversity and Adaptability of Bees

French beekeepers have imported honeybees from virtually the world over. Interracial hybrids, bred by artificially inseminating queens, can produce yields up to double those of the black honeybee (*Apis mellifera mellifera*) (Fresnaye et al. 1974). Royal jelly producers are working with foreign races because the French dark bee is highly productive (Jean-Prost and Le Conte 2005). A network for importing foreign queens has therefore been established in France for a very long time. However, these hybrids and other races are often less adapted and more susceptible to disease than local races. In France, there are therefore those who defend the local dark race, as these ecotypes are well adapted to their biotope, whilst others choose to import and use hybrid bees to ensure better harvests. The result of these opposing trends is wide genetic diversity in France, owing to continuing hybrid imports and to the genetic pollution of local honeybees.

In North America, it is prohibited to import queen bees for health reasons. In spite of the huge sums spent on preventing such imports, American honeybees have more diseases than European ones. In the USA, a few large-scale queen bee breeders are responsible for renewing the stock, with each breeder selling hundreds of thousands of queens. They raise their queens from just a few of their best strains, which reduces the genetic diversity of the honeybee population and so weakens the bees' defenses against various pathogens. In a global warming perspective, France's situation is assuredly a more comfortable one, as French honeybees are endowed with

greater genetic diversity and therefore have greater adaptive potential. The same is not necessarily true of other European countries that ban imports and select their stock rigorously.

This is an idea to be considered in the context of the spontaneous emergence of lines of *Varroa*-resistant honeybees, as has occurred in France with the emergence of honeybee colonies that have survived for more than 10 years without any treatment against the *Varroa* mite (Le Conte et al. 2007). However, countries that select their honeybees and import very few, such as Germany and the USA, have not yet detected any *Varroa* mite resistance in their honeybees. Molecular biology is a useful tool for measuring the genetic diversity of honeybee populations and linking it with their adaptability to climate change and to different pathogens (Ben-Shahar et al. 2006). Genomics is another useful tool that has become available following the recent sequencing of the bee genome (Weinstock et al. 2006), which will enable us to gain a better understanding of the coevolution mechanisms between honeybees and their pathogens (Navajas et al. 2008). A deeper understanding of these mechanisms will lead to better management of honeybee populations and the detection of the genes involved in new bee phenotypes.

19.9 Different Diseases in Different Parts of the World

Current disease profiles and potential changes in distribution arising from climate change suggest that some known pathogens are distributed worldwide. They include: *V. destructor* in the case of *A. mellifera* and *A. cerana*; bacteria that cause American and European foulbrood; *Nosema Apis* and *N. cerana*; and numerous viruses affecting *A. mellifera*. These pathogens tend to have different haplotypes of varying virulence. Climate change can encourage the transfer of these haplotypes to honeybee populations.

Other pathogens or haplotypes have more limited distribution ranges, such as *Tropilaelaps*, which to date has been found only in Asia (Sammataro et al. 2000). Climate change will lead to movements of honeybees of different species and races, bringing them into contact with pathogens with which they have never coevolved, as has occurred with *V. destructor* and *A. mellifera*. In the space of a few decades last century, two extremely homogeneous haplotypes of this honeybee parasite were sufficient to invade virtually the entire *A. mellifera* distribution range (Solignac et al. 2005). History therefore shows that such encounters can be catastrophic and that honeybees will need human assistance to survive. Honeybee movements may be spontaneous and linked to changes in geographical distribution, or the result of exchanges of bees among beekeepers. There could be changes in the geographical distribution of diseases whose expression depends on climatic factors. This has happened with chalkbrood disease, which is caused by the fungus *Ascosphaera Apis*, which develops mainly in a humid environment.

19.10 How Will the Pathogen–Bee Interaction Evolve?

Recent results from a metagenomic study by American researchers on honeybee populations suffering from colony collapse disorder are highly instructive in this respect. They have shown that honeybee colonies are infested by numerous pathogens, including imported ones. There is therefore a high likelihood that as yet unidentified pathogens exist on certain honeybee species or races.

Pathogen species infesting different honeybee races or species can be brought into contact with new hosts. The recent discovery of *N. cerana* (Higes et al. 2006) and the Israeli acute paralysis virus (Cox-Foster et al. 2007) among *A. mellifera* is a potent example of the role humans can play in movements of honeybee populations. Climate change could modify the interactions among these different pathogens. *Tropilaelaps* is an interesting case in point. The *Tropilaelaps* mite does not yet infest *A. mellifera* because this honeybee's development cycle includes a period without brood, on which the mite is utterly reliant for its survival (Sammataro et al. 2000). However, if climate change induces warmer winters, *A. mellifera* would have to adapt towards a continual brood cycle, which would render it a potential host for *Tropilaelaps*.

19.11 Consequences for Bee Health and Socioeconomic Impact

Honeybees will require human protection, if only because of their importance for agricultural production and markets. It seems clear that bees will come into contact with new pathogens. The high mortality rate and colony collapses that we are currently seeing demonstrate the fragility of honeybee populations worldwide. As has been the case with the *Varroa* threat to *A. mellifera*, our honeybees will need to be aided with medicines and appropriate control methods to prevent them from becoming extinct.

19.12 Climate Change Can Facilitate the Emergence of New Invasive Species

Numerous examples have revealed the fragility of the host–parasite balance and have shown that even slight climate changes impact the establishment of invasive species that are currently at the fringes of the honeybees' distribution range. The situation of honeybees can also evolve when predators colonize new areas. A stark example is that of the bee-eater, a magnificent bird that feeds on *Hymenoptera* and bees. The bee-eater originated in the Mediterranean region but has extended its distribution range, causing only minimal harm to beekeepers so far. In France it is now found north of the Loire. A second example is an apiary pest, the small hive beetle (*Aethina tumida*), which originated in South Africa and develops on the weakest honeybee colonies. The parasite was imported into the USA, probably on

citrus fruit on which the beetle can also develop. It has compounded the problems of American beekeepers, especially in hot and humid regions. The cold climate has halted the beetle's northward progression. Climate change will promote the extension of its distribution range. Measures have been taken to prevent this insect pest from being imported into Europe, where it is considered a potential hazard.

19.13 Socioeconomic Aspects

Not only bees, but beekeepers too, will need to adapt to changes in climate and flora. This means that some regions that are now hostile to beekeeping will become of interest to beekeepers, whilst other foraging areas will have to be abandoned. A decisive factor in beekeepers' choices will be the adaptiveness of honey plants to climate change. Beekeepers will also need to adapt their bees to climate change, abandoning local ecotypes or races in favor of better-adapted honeybees. This means that measures must be envisaged to conserve honeybee races and ecotypes to limit the loss of bee biodiversity. An appealing technique is sperm cryopreservation.

19.14 Recent Cases of Mortality

Since 1995, we have been seeing heavy mortality among *A. mellifera* worldwide. The consensus among researchers is that a combination of factors is responsible for this honeybee mortality. Pesticides kill many colonies every year. New pathogens have been added to the already long list of honeybee diseases. However, researchers agree that the bees' environment and stress, both of which are influenced by climate change, have been decisive factors in this heavy mortality (Oldroyd 2007; Pettis et al. 2007). There appear to be strong interactions between diseases, pesticides, environment, and climate. As climate change has an action on the evolution of honeybee populations, each of these factors will need to be taken into account.

19.15 Climate Effects and Plant–Pollinator Interactions

To understand how climate change is affecting plant–pollinator interactions and to predict future changes, the influence of climatic variables such as temperature and precipitation on plant and pollinator communities and their interactions along natural gradients may provide critical insight. González et al. (2009) found that rainfall was important in explaining variation in bee and fly interactions with plants. Similarly, precipitation was found to influence species prevalence and turnover in Patagonian plant–pollinator communities as well as the percentage of interactions established by each taxon (Devoto et al. 2005, 2009). In the West Indies, increased rainfall and decreased temperature with elevation are accompanied by phenotypic and ecological

specialization of plant species on hummingbird pollination (Dalsgaard et al. 2009). As water availability through patterns of rainfall and snowmelt can influence the timing and abundance of flowers and floral rewards (e.g., Inouye et al. 2003; Lambert et al. 2010), the potential links between increased frequency and intensity of droughts with climate change and subsequent effects on plant–pollinator interactions are clear (Alarcón et al. 2008).

Plants are very sensitive to the seasonality of their environment, and phenological shifts in plants provide undeniable evidence that climate change is affecting species and ecosystems (Cleland et al. 2007). For instance, over 500 plant taxa from Massachusetts, USA, first studied by Henry David Thoreau in the mid-nineteenth century, have been shown to be affected by global warming, with plants blooming earlier now than before (Miller-Rushing and Primack 2008). In addition to plants blooming earlier, warmer temperatures are also correlated with earlier activity in some insect pollinators (reviewed in Hegland et al. 2009). A point of special concern is that pollinators appear to be experiencing a greater degree of phenological advancement than plants, potentially leading to interaction mismatches.

Studies simulating the effects of climate change on plant–pollinator interaction webs provide expectations and hypotheses to test under observed or experimentally induced climate change. Memmott et al. (2007) modeled the potential effects of phenological shifts on plant–pollinator interactions, finding reduced or eliminated floral resource availability for many pollinators and thus potential extinction cascades. In addition to the temporal model of Memmott et al. (2007), Devoto et al. (2007) considered spatial movement of pollinators by simulating a range shift over a steep rainfall gradient, similar to predictions of decreased precipitation with climate change. In this model, they found plant–pollinator interaction networks to be fairly robust to these range shifts. Neither of these models account for behavioral flexibility of pollinator foraging. As species go extinct, alter their range distributions, or no longer overlap in phenology, pollinators may alter their behavior to forage on and pollinate new plant species. Thus, models that do not account for behavioral flexibility will likely overestimate the overall network effects of phenological change on plant–pollinator interactions.

Pollination ecologists have come a long way in describing the structure of plant–pollinator interaction networks; however, there is still much to do to better understand patterns and processes related to spatiotemporal variation in pollination systems. For example, additional long-term studies across diverse habitats are sorely needed given that the best available data set is from a ca. 4-year survey (Petanidou 1991) of a Phryganean community in Greece. These data have contributed significantly to our understanding of temporal patterns (Petanidou and Ellis 1993; Petanidou and Smets 1996; Petanidou et al. 1995, 2008; Medan et al. 2006; Petanidou and Potts 2006); however, the time frame is still too short to document the long-term effects of climate change, species introduction, or succession. To increase our understanding of long-term temporal patterns, pollination ecologists, or their students, could return to their former study sites at 5–10-year intervals or to sites of historic studies to resurvey plant–pollinator interactions and build upon existing data sets.

Animal pollination of both wild and cultivated plant species is under threat as a result of multiple environmental pressures acting in concert (Schweiger et al. 2010). Invasive species (Memmott and Waser 2002; Bjerknes et al. 2007), pesticide use (Kearns et al. 1998; Kremen et al. 2002), land-use changes such as habitat fragmentation (Steffan-Dewenter and Tschamntke 1999; Mustajarvi et al. 2001; Aguilar et al. 2006), and agricultural intensification (Tschamntke et al. 2005; Ricketts et al. 2008) have all been shown to negatively affect plant–pollinator interactions. Climate change may be a further threat to pollination services (Memmott et al. 2007; Schweiger et al. 2010; Hegland et al. 2009). Indeed, several authors (van der Putten et al. 2004; Sutherst et al. 2007) have argued that including species interactions when analyzing the ecological effects of climate change is of utmost importance.

In a recent review, Hegland et al. (2009) discussed the consequences of temperature-induced changes in plant–pollinator interactions. They found that the timings of both plant flowering and pollinator activity seems to be strongly affected by temperature. Insects and plants may react differently to changed temperatures, creating temporal (phenological) and spatial (distributional) mismatches—with severe demographic consequences for the species involved. Mismatches may affect plants by reduced insect visitation and pollen deposition, while pollinators experience reduced food availability. We have found three studies investigating how increased temperatures might create temporal mismatches between wild plants and their pollinators. Gordo and Sanz (2005) examined the nature of phenological responses of both plants and pollinators to increasing temperatures on the Iberian Peninsula, finding that variations in the slopes of the responses indicate a potential mismatch between the mutualistic partners. Both *A. mellifera* and *Pieris rapae* advanced their activity period more than their preferred forage species, resulting in a temporal mismatch with some of their main plant resources (Hegland et al. 2009). However, Kudo et al. (2004) found that early-flowering plants in Japan advanced their flowering during a warm spring whereas bumble bee queen emergence appeared unaffected by spring temperatures. Thus, direct temperature responses and the occurrence of mismatches in pollination interactions may vary among species and regions (Hegland et al. 2009).

19.16 Impact of Climate Change on Plant–Pollinator Interaction

Studies show that surface air temperatures in India are going up at the rate of 0.4 °C every 100 years, particularly during the post-monsoon and winter seasons (Ravindranath and Sathaye 2002). One of the effects of global warming on the plant community that was recognized was on the flowering phenology. Global climate change could significantly alter plant phenology because temperature influences the timing of development, both alone and through interactions with other cues, such as photoperiod. Pollination is an important ecosystem service considered as a regulating service by the Millennium Ecosystem Assessment. It is fundamental to the reproduction of flowering plants and is essential for the production of about one-third of the

food consumed by humans (Klein et al. 2007). Pollination is often taken for granted and is commonly thought of as a free service. Of the 352,000 species of flowering plants in the world about 87.5 % (i.e., 306,000 species) depend on animals, mostly insects, for pollination. Nearly 75 % of the world's food plants show increased fruit or seed-set with insect visitation (Klein et al. 2007). The economic value of this benefit is estimated to be about \$ 153 billion per year (Gallai et al. 2009).

Pollinators are considered keystone species in many situations not only because they support humanity but also maintain diversity in an ecosystem. There is mounting evidence of a global decline in pollinators that threatens the reproductive cycle of many plants and may reduce the quality and quantity of fruit and seeds, many of which are of nutritional and medicinal importance to humans. Such evidence includes the finding that those plant species that depend on pollinators which are declining have, themselves, declined relative to other plant species indicating a causal connection between local extinctions of functionally linked plant and pollinator species (Biesmeijer et al. 2007).

A growing number of studies suggest that climate change may be one of the biggest disturbance factors imposed on ecosystems today (Walther et al. 2002; Parmesan 2006). Observational evidence from many continents has indicated that many ecosystems are affected by regional and global climate changes, particularly temperature increases. Because of the cumulative evidence of a close relationship between greenhouse gas emissions through human use of fossil carbon and global change (IPCC 2007a), there has been a surge of scientific interest in the ecological and evolutionary effects of climate warming. Studies have shown that both the distribution and phenology of many plants and animals are biased in the directions predicted from global warming in the last few decades (Parmesan 2006).

For species involved in pollination interactions, this is evident through recent changes in flowering phenology (Fitter and Fitter 2002; Miller-Rushing et al. 2007) and the first appearance dates of butterflies and migrating birds (Roy and Sparks 2000; Gordo and Sanz 2005, 2006). It depends on how interactions among species are influenced whether climate warming will affect ecosystem functioning. Several studies have shown alterations in trophic relationships and energy-flows in both predator–prey and plant–herbivore interactions as a consequence of rising temperatures (Visser and Both 2005).

We should be concerned about climate change in India, since this phenomenon might have substantial adverse impacts on flowering phenology of cultivated crops, their pollinators, and in short, on the productivity of such crops. The impact on agriculture would be unimaginably high through the effects of climate change on pollinator populations. However, the information available on pollinator decline due to climate change and the consequent effect on agricultural production in India is very scanty. Phenological networks rely on volunteers to collect observations of various phenophases of wild plants, fruit trees, and agricultural crops at numerous stations. The longest and best known phenological records come from the Far East and Europe, including a 5,000-year record of phenological events, weather, and farming activities in China (Chen 2003), the 1,300+-year Kyoto cherry blossom time series

(Menzel and Dose 2005), 670+ years of grape harvest dates in Central Europe, and the 200+-year Marsham record of plant and avian phenology in the UK (Sparks and Carey 1995). More recently, shifting phenology from the mid-twentieth century onwards is evident from numerous phenological observation networks in the Far East (Matsumoto et al. 2003), North America (Schwartz and Reiter 2000), and Europe (Menzel et al. 2006). A meta-database of existing networks shows that most observation networks are located in temperate ecosystems and that long-term phenological observations in the tropics are lacking. The timing of pollination is determined by climatic cues such as temperature and water availability (Cleland et al. 2007). Many pollinators also synchronize their life cycles with climatic cues, and this phenological response of plants and pollinators needs to remain broadly synchronized for many plant–pollinator relationships to remain viable. Climate change is altering the phenological response of plants and some pollinators may be unable to alter their life cycles in synchronization with altered pollination timing. Kudo et al. (2004) show that, since 1998, plants have been flowering much earlier in alpine environments whilst the time of emergence of pollinators has not necessarily changed, thereby disrupting pollination. Climate change also shifts the latitudinal and altitudinal climate “envelope” of species. Some species are more mobile or adaptable to change and so the composition of plant and pollinator assemblages is likely to change in many locations. For example, species in the tropics appear to be living at or near their thermal optimum and further warming may cause some species to migrate to cooler areas or die out (Deutsch et al. 2008).

Any decline in pollination services could lead to cascading effects on ecosystems and an overall loss of biodiversity and ecosystem services (such as agricultural production). Mutualistic relationships are most directly affected since the loss of individual pollinator species will lead to the extinction of any co-dependent plant species (and vice-versa). A study conducted under the European project Assessing Large-scale environmental Risks for biodiversity with tested Method (ALARM) has shown that specialist pollinators and the obligate out-crossed plants that they pollinate decline in tandem (Biesmeijer et al. 2006).

Ecosystems possess some robustness to the loss of individual species since multiple pollinators can pollinate most plants, each with somewhat different effectiveness or responses to environmental change. However, the loss of particular pollinator species and the impoverishment of pollinator diversity diminish the resilience of ecosystems to change, which are subsequently less able to provide services to humans (FAO 2008a). Many pollinators are also important food sources for higher animals, so their loss may threaten birds, bats, and other small mammals. As individual species are lost from an ecosystem, the functional redundancy that diverse ecosystems generally display is reduced and resilience to change also tends to decline.

Though there have been extensive studies on effects of climate change on agricultural crops, none, whatsoever, have concentrated on the effects on pollinator populations and the consequent reduction in agricultural productivity in India except for a few anecdotal references on the shifts in apple cultivation to higher altitudes in Himachal Pradesh. Historical data on flowering phenology is available for many

countries for various crops but in India we may get this kind of information only for coffee and a few spice crops, to help us compare with the past weather parameters.

Climate influences flower development and nectar and pollen production, which are directly linked with the colonies' foraging activity and development (Winston 1987). A major effect of climate change on honeybees stems from changes in the distribution of the flower species (Thuiller et al. 2005) on which the bees depend for food. Rain can impact honey harvesting. For example, when acacia (*Acacia* spp.) flowers are washed by rain, they are no longer attractive to honeybees as it dilutes their nectar too much (Conte and Navajas 2008). Likewise, an overly dry climate can reduce the production of flower nectar for honeybees to harvest, since many plant flowers produce no nectar when the weather is too dry, which makes harvesting by bees a largely hypothetical matter. In these situations, honeybees can die of starvation. Climate change presents an additional major challenge to indigenous people and rural communities which brings with it new problems, often interacting with or exacerbating existing problems. It makes new demands for adaptation and coping strategies by farmers and rural communities and presents new challenges for the management of their environment and agro-ecosystems.

The Hindu Kush Himalayan (HKH) region is blessed with a diversity of honeybee species; five species—*Apis cerana*, *Apis dorsata*, *Apis florea*, *Apis laboriosa*, and *Apis andreniformis*—are indigenous, while *A. mellifera* is introduced. Of these species, only *Apis cerana* and *Apis mellifera* can be kept in hives and managed for honey production and pollination. Challenges arising from climate change are affecting bee populations and the flowering of crops and other plants that provide nectar and pollen for colony development and honey production. The ecosystem services rendered by pollinators are of great importance providing not only pollination of useful plants but also, in the case of bees, useful products and income. Plant species diversity in agricultural ecosystems is also often crucial for the maintenance of the birds, bats, and insects that are the principal pollinators of many crops (McNeely and Scherr 2001). During the past few years, apple production in Himachal Pradesh in the north-west Indian Himalayas of Nepal has been declining continuously. A study conducted by the Beekeeping Project of ICIMOD has shown that this decline in productivity is due to pollination failure. The reasons are lack of pollinizers (i.e., trees that can provide fertile compatible pollen) and lack of pollinators (bees, butterflies, and moths). To overcome the lack of insect pollinators, farmers are renting honeybees (*A. cerana* and *A. mellifera*), decreasing the numbers of pesticide sprays, and carrying out hand pollination. It is reported that climate change during the past 8 years has played a critical role in apple pollination failure. There are rains during the flowering season which affect pollination by wind and insects. Low temperatures also affect fruit set in apples adversely. The climatic changes alter the pattern of blossoming, bearing and, therefore, fruit yield. The lack of early cold in December and January is understood to affect the chilling requirements adversely, which range from 700 to 1200 h/year. As Abbott (1984) points out, a chilling requirement averaging 10 weeks below 5 °C is required to meet the internal conditions necessary for bud-break with the onset of spring temperatures. An April late cold can delay blossoming and reduce the pollination activity of bees. Also, if it rains in this period, there is a risk of pollen

being washed away from plants. The amount of snow is understood to strongly influence soil moisture, especially in the case of early snow. Late snow, besides having a negative impact in several other aspects, also fails to replenish soil moisture to the desired degree. In addition, late snow affects the process of pollination indirectly; a relative immobilization of bees is triggered due to low temperatures brought about by late snowfall. Himalayan honeybee *A. cerana*, endemic to the area, starts foraging at temperatures as low as 7 °C, whereas *A. mellifera*, which has been introduced over the last 10 years, begins at around 13 °C (Pratap and Pratap 1997)

The ecological effects of climate change are proving to be extensive and affect the life histories, phenologies, distributions, species interactions, and survival of a great diversity of organisms. The combination of abiotic factors defined as climate is an important variable in the distribution of species (Grinnell 1917; MacArthur 1972) because changes in the abiotic characteristics define the fundamental niche of a species (Kearney and Porter 2004). As climate change has continued to occur, changes in ecosystems have become especially apparent across altitudes. Observations have occurred on all scales from the spatial shifts in distribution of various plant and animal species to the arrival date of migratory birds (Parmesan 2006; Siano et al. 2011). However, most of the range shifts that have been observed recently have occurred across altitude. For example, in mountainous regions, the tree line has advanced to higher elevations as temperatures have increased over the past few decades (Jump et al. 2009). In general, an increase of 1000 m in altitude correlates with a decrease in ambient air temperature of approximately 6 °C. As the climate has continued to warm, temperatures have increased over all elevations, so the temperature gradient across altitude is still intact, though overall temperatures at any altitude are higher now than they were in the past (IPCC 2007b).

19.17 Phenology and Climate Change

Phenology, the timing of various events in a species' life cycle, is an important life history trait for both plants and animals. In angiosperms, an important portion of the temporal niche is the time during which it is optimal for them to flower (Bronstein et al. 1990). During this time, pollinators should generally be abundant and there should be low competition with other flowers for pollinators. However, depending on the environment, flowering of other species at the same time might cause competition (Waser 1978) or facilitate pollination due to attraction (Tachiki et al. 2010).

Observations of changes in the phenology of plants in response to climate change over the past few decades are numerous. In addition to changing their spatial distributions, plants are also changing their temporal niches (e.g., Miller-Rushing and Primack 2008; Miller-Rushing and Inouye 2009). Important plant events such as spring bud burst and flowering are occurring earlier in many locations, and appear to be a result of earlier snowmelt and higher temperatures (Parmesan 2006). Plants use these abiotic indicators in order to determine what day to put out leaves, when to produce chlorophyll, or when to flower. As a result, spring and summer events

in a plant species' life cycle have tended to advance as temperatures have warmed. For instance, the first, peak, and last flowering dates of the alpine species *Erythronium grandiflorum* have advanced an average of 3.2 days per decade over the past 35 years (Lambert et al. 2010). The phenology of this species is largely controlled by snowmelt date, which has advanced as temperatures have increased.

However, perhaps due to the diversity of factors that plants use to determine their phenologies and their diversity of niches, different plant species alter their phenologies at different rates in response to climate change. According to studies conducted to date, snowmelt generally controls the flowering phenology of subalpine plants, and plants have a shorter time to develop and flower in these harsh conditions (Inouye et al. 2002). In the Rocky Mountains, USA, early flowering species' phenologies, in particular, have demonstrated a stronger correlation to snowmelt date to snowmelt than late-flowering species (Dunne et al. 2003; Miller-Rushing and Inouye 2009). Subsequent research has suggested that such dependence upon snowmelt date is species dependent (Forrest et al. 2010). Other factors affecting the timing and abundance of flowering in subalpine environments are changes in precipitation (Crimmins et al. 2010; Lambert et al. 2010) and temperature (Hülber et al. 2010). Overall, broad trends suggest that early-flowering species are reacting more drastically to the rising temperatures than are late-flowering species (Miller-Rushing and Inouye 2009).

When different plant species react to changes in climate at different rates, there is an increased risk of changes in the flowering plant community's phenology. An especially important part of flowering plant phenology is co-flowering. Co-flowering is the phenology of when different plant species overlap in their flowering (Forrest et al. 2010). Co-flowering is ecologically important in plant communities because it determines interspecific interactions that will occur that directly affect plants' reproduction (Lázaro et al. 2008). Only plant species that flower at the same time will be competing (or cooperating) to attract pollinators. Surprisingly, few studies have examined the link between changes in altitude and the flowering phenology of subalpine plants. Climate—temperature, precipitation, snowmelt date, etc.—changes dramatically with temperature, and one would expect a corresponding change in phenology as altitude changed. However, most research to date has focused on changes in timing as temperatures have risen at a single location (Price and Waser 1998; Inouye 2008).

Studies to date of changes in phenology across altitude have tended to occur in montane and alpine regions. In Argentina, temperature appears to influence the phenology of bud burst and flowering of *Nothophagus pumilio* in montane forests; bud burst and flowering were both delayed at higher altitudes where temperatures were colder (Rusch 1993). Once again, it appears that altitude was not the factor directly affecting phenology, but temperature, which co-varies with altitude, was the important factor. In Great Britain, researchers found that the hatching date and emergence of adult spittlebugs delayed as altitude increased across a 440 m gradient (Fielding et al. 1999). Fielding et al. (1999) suggested that such important changes in the phenology of these insects are likely to affect them and their interactions with other trophic levels. In contrast, a study of several plant species across the European Alps did not find that plant phenology significantly changed as a function

of altitude, though phenology did change as temperatures warmed over the past few decades (Ziello et al. 2009). The inconsistency of phenological change across altitude implies that the factors affecting phenology are diverse; some of those factors covary with altitude while others do not. The diversity of drivers of phenology indicates that changes in factors across altitude could have significant effects on community dynamics. Crimmins et al. (2010) in the arid American southwest found that plant species had highly individualistic responses to climate change over a 20-year period; furthermore, altitude also had an important effect on phenological response. Such variability could have far-reaching consequences for the function of an ecosystem and the distribution of flowers.

19.18 Impact of Climate Warming on Flowering Plants and Pollinators

Many organisms respond to changes in temperature by altering their activity and metabolism. Therefore, anthropogenic induced temperature increases have the potential to affect the phenology of both plants and pollinators. Until recently, the strength and direction of the phenological responses to increasing temperatures was mainly unknown. Indeed, it had not been shown whether phenological shifts occurred at all in natural communities in response to climate change. However, in the last decade there has been increasing interest in phenological responses to climate warming (Post and Inouye 2008; Rosenzweig et al. 2008) and much of the knowledge on climate warming effects comes from phenological research.

Onset of flowering is regularly used to measure the arrival of spring in temperate habitats. Many plants appear to have reacted to increasing temperatures by earlier flowering during the last 20–50 years (Fitter and Fitter 2002). In Europe, 78 % of the observed time series provided such a trend (Menzel et al. 2006), which concurs with findings from other parts of the northern hemisphere (Sparks et al. 2000; Miller-Rushing et al. 2006, 2007). Insect-pollinated plants generally react more strongly to increased warming than wind-pollinated plants, and species flowering early in the season appear to be most sensitive (Fitter and Fitter 2002; Miller-Rushing et al. 2007), an indication that these species have thermal-sensitive phenologies. In general, the onset of flowering appears to be correlated with the mean temperature in the month of flowering or the months prior to flowering (Sparks et al. 2000; Menzel et al. 2006). However, other factors may co-vary with temperature and be important for the observed patterns. The responses of flowering onset to increasing temperatures were linear in most cases (Sparks et al. 2000; Fitter and Fitter 2002; Gordo and Sanz 2005; Menzel et al. 2006), which could be important for plant interactions with pollinators.

Although it is obvious that such linear responses cannot continue perpetually, Sparks et al. (2000) found significant linearity within the observed range of temperature variations for 24 of 25 British plant species of which 23 flowered earlier

with increased temperatures. To understand the impacts of climate warming better, the already mentioned generalizations of species' responses are important. We emphasize, however, that some species may not flower earlier as a response to increased temperatures (e.g., 22 % of the species included in Menzel et al. (2006)). Also, other potential cues for flowering initiation include photoperiodicity, precipitation, soil humidity, and snow melt (Inouye et al. 2003; Price and Waser 1998) as well as a particular combinations of cues (e.g., Lambrecht et al. 2007). If climate change disrupts the relationships among the environmental cues which plants use to initiate flowering, past combinations of cues might reappear at novel times in the season (Price and Waser 1998; Stenseth and Mysterud 2002; Visser and Both 2005), resulting in "bizarre" flowering times. Furthermore, phenological responses of plant species to previous temperature increases do not indicate whether plants' future responses to temperature will continue as linear, level off, or follow some other relationship (Sparks et al. 2000). Such future responses not only depend on a plant species' direct response to temperature or other cues, but may be modified ecologically, or evolutionarily, by the interactions with its pollinators (Fig. 19.2).

Flowering duration is another phenological aspect of great significance, both for plant reproduction and pollinator food supply. There is clear evidence for prolonged growing seasons in many plant communities in Europe during the last decades (Menzel and Fabian 1999), but the length of the flowering season appears less affected, especially for later-emerging species that show a more variable response to climate warming (Miller-Rushing and Primack 2008). For example, warming experiments in alpine regions have found both indications for prolonged flowering (Dunne et al. 2003) and no change in flowering duration (Price and Waser 1998).

Most pollinators are insects and, because insects are small and poikilothermic, it is likely that temperature will be critical for their life cycle development and activity patterns (Fig. 19.1), which is particularly evident in alpine and arctic regions (Totland 1994; Hodkinson et al. 1998). Butterflies are appealing organisms frequently attracting public attention.

As a result, long-term butterfly monitoring programs exist in several countries and studies based on such data have documented a close positive relationship between first appearance date and temperature (Roy and Sparks 2000; Forister and Shapiro 2003). Across climatic zones in Europe, the date of first emergence of butterflies is strongly correlated with temperatures in the month of, or previous to, appearance (Roy and Sparks 2000; Gordo and Sanz 2006). Roy and Sparks (2000) also showed that peak appearance of butterflies came earlier and flight duration was prolonged during a warming period from 1976 to 1998, both variables potentially influencing the interactions with the plants they pollinate.

Bees are the most important pollinators for many wild and cultivated plant species. Long-term data from Spain, spanning more than 50 years, show a clear relationship between the first appearance dates of *Apis mellifera* L. (the honeybee) and early spring temperatures (Gordo and Sanz 2006). Honeybees can be considered good indicators of climate change as they overwinter in the adult phase, and appear to react quickly to increases in spring temperatures (Gordo and Sanz 2006). Likewise, data from Nature's Calendar project (available online at <http://www.naturescalendar.org.uk>)

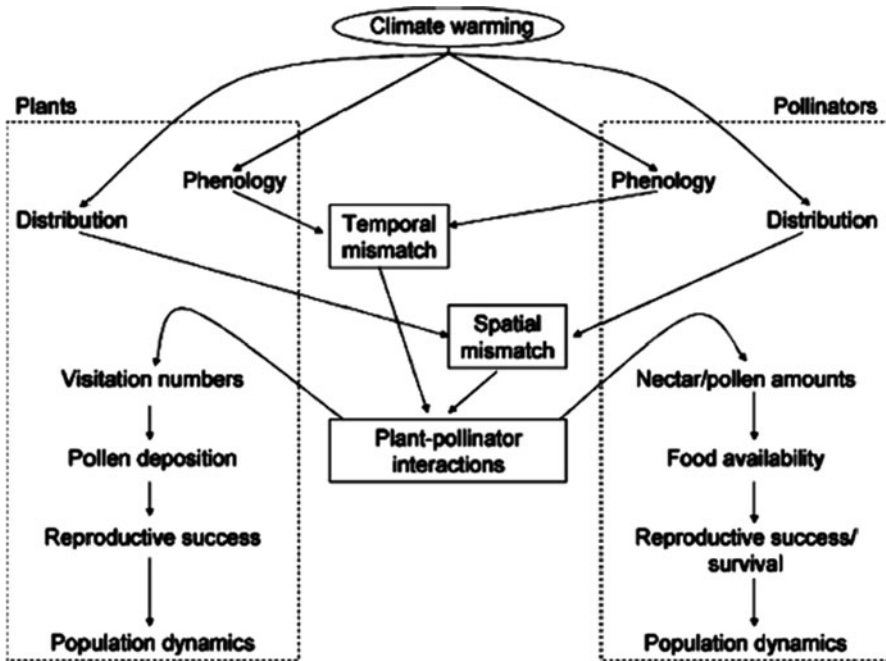


Fig. 19.1 Framework showing how climate warming may affect the phenology and distribution of plants (*left panel*) and pollinators (*right panel*) thereby creating temporal or spatial mismatches in plant–pollinator interactions. In the *lower half* of the panels we show how and by which key factors the demography of the mutualistic partners are likely to be affected. The pathway until the mismatches is largely known, whereas the mismatches and the subsequent effects are still mostly unknown and requires additional research. (Redrawn from Hegland et al. 2009)

indicate that bumble bees have advanced their spring flight times by 2 weeks from 2001 to 2007 (Sparks and Collinson 2007), probably caused by higher soil temperatures that terminate queens’ winter hibernation (Alford 1969). As for plants, available data indicate a linear relationship between temperature and pollinator phenology and that the effect is strongest in early-season events within the span of observed temperature changes (Roy and Sparks 2000; Forister and Shapiro 2003; Gordo and Sanz 2005, 2006; Menzel et al. 2006; Fig. 19.2).

19.19 How Does Climate Warming Affect Plant–Pollinator Interactions?

Climate warming affects the phenology, local abundance, and large-scale distribution of plants and pollinators. Despite this, there is still limited knowledge of how elevated temperatures affect plant–pollinator mutualisms and how changed availability

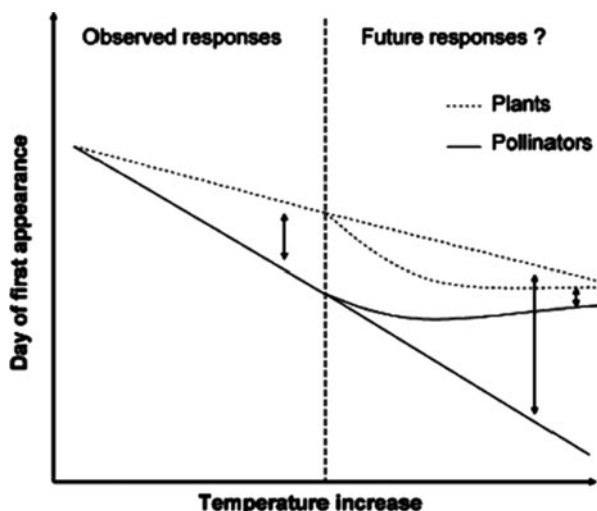


Fig. 19.2 The linear relationships between climate warming and appearance dates in pollinators (flight activity) and plants (flowering) as currently observed, and some potential future development of this relationship. The nature (i.e., linear response) and the magnitude (slope) of responses (i.e., strongest in pollinators) are based on the few available studies (e.g., Gordo and Sanz 2005), and must therefore be viewed as an approximation of how climate warming may shape temporal plant–pollinator mismatches. The length of the *arrows* visualizes the relative magnitude of temporal mismatch between plant and pollinator. The *lines* connected by the *short arrow* (under future responses) illustrate one possible future situation where the adaptation of plant and pollinators to future climates are influenced by the interaction among them and the mismatch is dampened. The *lines* connected by the *long arrow* shows an extrapolated projection of the general current trend where phenologies are mainly influenced by temperature resulting in a potential further increase in the mismatch

of mutualistic partners influences the persistence of interacting species. Observational evidence from all continents shows that many ecosystems are affected by regional and global climate changes, particularly temperature increases (IPCC 2007b). A growing number of studies suggest that climate change may be one of the biggest anthropogenic disturbance factors imposed on ecosystems today (Walther et al. 2002; Parmesan 2006). Because of the cumulative evidence of a close relationship between greenhouse gas emissions, through human use of fossil carbon, and global change (IPCC 2007b), there has been a surge of scientific interest in the ecological and evolutionary effects of climate warming. Studies have shown that both the distribution and phenology of many plants and animals are biased in the directions predicted from global warming in the last few decades (Parmesan 2006), indicated by a global advancement of spring events by 2.3 days per decade and a species range shift of 6.1 km per decade towards the poles (Parmesan and Yohe 2003). For organism groups involved in pollination interactions, this is evident through recent changes in flowering phenology, e.g., onset of flowering (Sparks et al. 2000; Fitter and Fitter 2002; Miller-Rushing et al. 2006) and the first-appearance dates of butterflies and

migrating birds (Roy and Sparks 2000; Gordo and Sanz 2005, 2006). It depends on how interactions among species are influenced whether climate warming will affect ecosystem functioning. Several studies have shown alterations in trophic relationships and energy-flows in both predator–prey and plant–herbivore interactions as a consequence of rising temperatures (e.g., Stenseth and Mysterud 2002; Visser and Both 2005; Durant et al. 2007).

Pollination interactions are important as they benefit both biodiversity and humans. A great diversity of plants and animals—mainly insects, but also some birds, lizards and mammals—depend mutually on each other for pollination and food, and their interactions may influence population persistence. There has been a growing appreciation of the importance of the ecosystem services provided by pollination interactions (e.g., Allen-Wardell et al. 1998; Ricketts et al. 2004; Klein et al. 2007), and it has been suggested that we may be in the middle of a global pollination crisis (Steffan-Dewenter et al. 2005; Biesmeijer et al. 2006), but also questioned (Ghazoul 2005). Recent reviews have indicated that knowledge of the effects of climate warming on mutualistic interactions is still limited (Walther et al. 2002; Visser and Both 2005), and that the current interest in pollination interactions has not yet resulted in much empirical research on how climate warming may affect this ecosystem service (Kremen et al. 2007; Memmott et al. 2007). Speculations on the disruptions of plant–pollinator interactions due to climate change are often brought forward (e.g., Harrington et al. 1999; Visser and Both 2005; Parmesan 2006), but few empirical studies exist to verify whether such disruptions do occur and reports still appear largely anecdotal.

19.20 Abundance and Distribution

Temperature-driven changes in flower abundance may have a large impact on pollination interactions. Increased reproductive effort (i.e., number of flowers) appears to be one commonly observed response to experimental warming in the arctic and alpine (e.g., Arft et al. 1999). On the other hand, observational studies have shown that increasing spring temperatures may decrease flower abundance (Inouye et al. 2003) and affect species' abundance in contrasting ways within a community (Tyler 2001). In both of these studies, the temperature effect is weaker than in warming experiments, probably due to interactive effects of precipitation and humidity. Mass flowering, i.e., masting, is also generally positively affected by high temperatures (Schauber et al. 2002). Increased flower abundance within a community may affect the reproductive success of plant species, for example, through increased visitation rates as a result of altered pollinator behavior and composition of the pollinator community ultimately increasing out-crossing rates and seed production (e.g., van Treuren et al. 1994; Hegland and Totland 2005). Conversely, increased flower numbers on individual plants may cause higher selfing rates due to increased geitonogamy (e.g., Vrieling et al. 1999).

Increased flower numbers may also affect pollinators, as food availability appears to be one of the most important factors governing the activity and population density of many pollinator species (Steffan-Dewenter et al. 2002; Westphal et al. 2003; Hegland and Boeke 2006; Steffan-Dewenter and Schiele 2008). Evidence of direct temperature mediated effects on the abundance of pollinators is relatively rare. However, information from pollination studies along altitudinal or latitudinal gradients (proxies for temperature influence) may infer how pollinator assemblages could be affected by climate warming. For example, flies appear to become more abundant in colder and wetter areas whereas bees are often more abundant in warmer and drier habitats (Arroyo et al. 1982; McCall and Primack 1992; Totland 1993; Lázaro et al. 2008), suggesting that the composition and dominance of pollinator assemblages may change with climate warming.

Climate is a strong determinant of the geographical range of species (Moen 1999; Fig. 19.1) and, although precise information of species' geographic range is relatively difficult to obtain, some generalizations about climate warming effects can be made. Large climatic oscillations have occurred during historical time and have caused changes in species distributions. For example, during major glaciations, species distributions compressed towards the Equator and descended from the mountains, while during warmer inter-glacial periods, species migrated toward higher latitudes and altitudes (e.g., Taberlet et al. 1998; Hewitt 2000). Recent range expansions of lepidopterans and treeline dynamics show similar patterns; they are moving towards higher latitudes and altitudes as forecasted by climate warming scenarios (Parmesan 2006). Kelly and Goulden (2008) showed that nine out of ten dominant plant species on a Southern California mountain had made a mean (+65 m) elevational shift upwards the last 30 years. In Norway, alpine vascular plants have shown altitudinal expansions and increases in abundance and diversity during the last century (e.g., Klanderud and Birks 2003).

19.21 Mismatches Between Plants and Pollinators

Plant–pollinator interactions can be disrupted in at least two ways; through temporal (phenological) and spatial (distributional) mismatches that may change the availability of mutualistic partners (Fig. 19.1). Mismatch occurs when the original mutualistic partners experience reduced sharing of habitat either in time or space, leading to a partial or complete trophic decoupling (Stenseth and Mysterud 2002; Visser and Both 2005). Memmott et al. (2007) simulated how global warming might affect a highly resolved plant pollinator network. They found that, depending on the phenological shifts applied, between 17 and 50 % of all pollinator species suffer from disruption of food supply due to temporal mismatch. They showed that specialized pollinators were most likely to be left with no food plants, but that generalist pollinators could also experience considerable diet reductions following phenological shifts. The variation across species in phenological responses to climate warming may also uncouple many plant–pollinator interactions because the pollinators cannot track all

their ancestral hosts, if some flower earlier and some later (e.g., Menzel et al. 2006; Memmott et al. 2007). As many pollinators visit plants quite opportunistically (e.g., Petanidou et al. 2008), such a phenological decoupling may result in the emergence of novel plant–pollinator interactions. Gordo and Sanz (2005) examined the nature of phenological responses of both plants and pollinators to increasing temperatures on the Iberian Peninsula, investigating the slopes of the responses as indications of a mismatch between the mutualistic partners. They found that both *A. mellifera* and *Pieris rapae* advanced their activity period more than their preferred forage species, resulting in a temporal mismatch with some of their main plant resources. This result agrees with how herbivorous insects respond to climate warming in relation to food-plant availability; insect phenologies advance more than plant phenologies (Visser and Both 2005; Sparks and Collinson 2007; Fig. 19.2). In contrast, Kudo et al. (2004) found that early flowering plants in Japan advanced their flowering during a warm spring, whereas bumble bee queen emergence appeared unaffected by spring temperatures resulting in a decreased seed-set in bumble bee-pollinated plants. Thus, direct temperature responses and the occurrence of mismatches in pollination interactions may vary among species and regions.

We know of no reports that have shown climate-driven spatial mismatches in pollination interactions, and there is less evidence of such spatial mismatches in ecological interactions in general compared to temporal decoupling (Devoto et al. 2007; Durant et al. 2007; Schweiger et al. in press). However, Devoto et al. (2007) simulated climate-driven range shifts along a rainfall gradient and found that relatively few species would go extinct and that the pollination systems appeared resilient to climate changes (here, rainfall), even when assuming that pollinators had no flexibility in host plant utilization and a total dependence of plants on pollinator visitation for reproduction and population persistence.

19.22 Consequences of Mismatches

Synchronized timing of mutualistic partners may be important for efficient pollination of plants and survival of pollinators (Fig. 19.1). Therefore, one of the major concerns related to global warming and pollination interactions is the demographic consequence of mismatches between plants and pollinators. Assessments of how pollination interactions might respond to climate-driven mismatches must be speculative, because little is known about how warming affects the demography and population dynamics of the involved partners (Fig. 19.1).

Furthermore, the effect of climate-driven changes in food (for pollinators) and pollinator availability (for plants) is difficult to predict because our knowledge on the relative importance of bottom-up and top-down forces in population regulation is still poor (e.g., Steffan-Dewenter and Schiele 2008). If mismatches are to seriously affect pollinator demography, pollinator population densities and distributions must be controlled by bottom-up forces (*sensu* Durant et al. 2007), such as flower abundance. Likewise, whether mismatches will significantly influence plant demography

depends on the extent to which plants are top-down controlled through effects of pollinator abundance on pollen availability and mobility (*sensu* Elzinga et al. 2007).

In plants, a mismatch with important pollinators could reduce pollen deposition through altered visitation (quantity or quality of floral visits), potentially increasing pollen limitation (Fig. 19.1). Among plant species, limitation of reproduction due to insufficient pollination is common (Ashman et al. 2004). However, the impact of pollen limitation (i.e., a top-down force) on population dynamics, and its relative importance compared to resource limitation (i.e., bottom-up forces), is still poorly understood, although a few studies have shown that increased seed-set or seed mass after supplemental pollination can positively influence recruitment, survival, and population growth rates of flowering plants (Hegland and Totland 2007; Price et al. 2008). Another consequence of mismatches is the cascading effects they might have on species interactions occurring later in the season. A crash in early-emerging pollinator populations may affect both early and later flowering species and sequentially flowering species may facilitate each other through maintenance of pollinator populations (Waser and Real 1979). Moreover, in northern regions many plants depend on bumble bees for sufficient pollination. If nest development is restricted by a mismatch between early emerging bumble bee queens and their main food plants, such early season events may influence pollination services later in the season.

In pollinators, we can expect that a mismatch with important forage species primarily will reduce food accessibility through altered availability of carbohydrates (nectar) and proteins (pollen), subsequently affecting pollinator survival and reproduction (Boggs and Ross 1993). The effects of mismatches on pollinator population dynamics may be more severe than with plants, because pollinator dependence on nutrition is often more absolute than flowering plant species dependence on pollination. For example, the reproductive success of hummingbirds may be determined by the degree of matching (both timing and peak abundance) with its main flower resources (Waser 1976). In such cases, the potential for bottom-up control on pollinators' population dynamics appears evident. The generally shorter life span of pollinators, especially insects, compared to plants makes them more sensitive to climate variability (e.g., Morris et al. 2008), and this may be one reason why the population dynamics of many pollinators vary profoundly in time and space (e.g., Williams et al. 2001).

Knowledge of the effect floral abundance (i.e. resource availability) has on pollinator population dynamics appears limited, but a recent study by Steffan-Dewenter and Schiele (2008) showed that bottom-up forces, including food availability, were more important than top-down forces (such as rate of parasitism) in regulating the population dynamics of a solitary bee species. An extended flowering period or increased food availability per flower in response to higher temperatures (e.g., Petanidou and Smets 1996) may partly compensate for diet reductions due to mismatches in time and space. Importantly, it is not only the presence or absence of an interacting species that determines the output of a mismatch, but also their abundance, because most plants and pollinators are generalists utilizing several mutualistic partners and many mismatches are only partial (e.g., Memmott et al. 2007). Altered abundance of the

interacting species can potentially compensate for or strengthen the consequences of trophic mismatches (Durant et al. 2007).

19.23 Buffers Against Mismatches

Despite potential negative effects of mismatches, there are also innate properties found in plant–pollinator interactions that might buffer against their impacts. One prolific study approach in the last decade has been to view ecological communities and systems as networks. Pollination ecology has, by studying entire plant–pollinator systems, been able to illustrate some of the robustness that exists in natural systems of mutualistic interactions. Plant–pollinator networks are very heterogeneous with the bulk of species having relatively few interactions, whereas a few species have many more interactions than expected by chance (Jordano et al. 2003; Vázquez and Aizen 2003). Overall, there are more generalist species in pollination networks than previously thought and strict one to one specialist relationships are rare in nature (Waser et al. 1996). This generalist tendency in pollination interactions may itself ensure that most species are not severely affected by climate-driven mismatches. Moreover, plant–pollinator networks display a nested structure, where a core of generalist species interact with each other, while most specialists interact only with these generalists (Bascompte et al. 2003). Furthermore, most pollination interactions are highly asymmetric, meaning that if a plant is very important to a pollinator (a high percentage of the pollinator's visits are to this particular plant), the importance of this pollinator to the plant is low (a low percentage of the visits received by the plant comes from this pollinator; Bascompte et al. 2006). The nested structure and predominantly asymmetric nature of the interactions within plant–pollinator networks have been shown to stabilize these systems and make them less sensitive to species extinctions, disturbance, and habitat loss (Jordano 1987; Memmott et al. 2004; Fortuna and Bascompte 2006). Such perturbations may create mismatches that resemble those expected after climate change. Regardless of these buffering properties, loss of generalist plant species in particular, may put other species of pollinators and plants at higher risk for extinction (e.g., Memmott et al. 2004). Studies on variations in plant–pollinator network properties through time have only recently started to appear (e.g., Olesen et al. 2008; Petanidou et al. 2008; Alarcón et al. in press). Knowledge of such temporal variation in plant–pollinator interactions is crucial for understanding how these systems are affected by altered climatic conditions. The cited studies all show that plant–pollinator interactions are highly dynamic and both the species comprising the networks and the links change dramatically through time. One consequence of this variation is that plant–pollinator systems may be robust against both temporal and spatial mismatches between pairs of species. The dynamic structure of the mutualistic networks might therefore act as a buffer against cascading effects of loss of species and links within the network, although mutualistic networks could also reach a tipping point and collapse under severe disturbance (Memmott et al. 2004; Fortuna and Bascompte 2006).

19.24 Evolutionary Responses to Mismatches

The phenology of species has evolved to match environmental conditions. It is, however, reasonable to believe that plants and pollinators may have different mechanisms underlying their phenology and respond to different environmental cues (Visser and Both 2005). Studies suggest that the activity of both plant and pollinating insects show linear relationships with current increases in temperature (e.g., Sparks et al. 2000; Gordo and Sanz 2005). Despite this apparent similarity in responses, the magnitude (i.e., the slopes) of responses may diverge, resulting in a mismatch (Fig. 19.2). A central question is whether the responses of plants and pollinators to increasing temperatures will be parallel or whether the partners will halt or accelerate their future response relative to the other (Fig. 19.2). The answer lies in the potential for adaptation and whether such adaptations will be driven mainly by temperature or whether the interaction itself may shape the organisms future responses. Figure 19.2 gives some possible future developments of species' phenological responses. Temporal mismatches among plants and pollinators may alter selection pressures that plants and pollinators exert on each other, and result in rapid evolution in pollination and reproductive traits in plants and foraging and phenological traits in pollinators as indirect responses to climate warming. A pre-requisite for such rapid genetic change is a sufficiently large fitness consequence of the experienced mismatch (Davis et al. 2005; Skelly et al. 2007). Moreover, to what extent climate warming affects selection pressures depends on whether the species use temperature as cues to track changes in the phenology of their mutualistic partners (see also the section on Future Research). Rapid evolution in response to climate change has been documented for both insects and flowering plants (Davis et al. 2005; Franks et al. 2007; Skelly et al. 2007). Species' generation times may be decisive for their potential to respond to rapid direct or indirect changes of climate warming (Davis et al. 2005). Thus, plant species with contrasting life histories may respond with different speed and pollinators, which generally have shorter generation times, may evolve faster as a response to changing climate conditions than their food plants (see also Morris et al. 2008).

19.25 Estimating Consequences of Mismatches

Species may, as mentioned above, not only react directly to climate warming, but also indirectly via changes in the availability of their mutualistic partners. A prime priority for future research should be to examine whether climate-driven temporal and spatial mismatches between plants and pollinators do occur and subsequently examine the potential ecological and evolutionary consequences of such mismatches (Fig. 19.1). Elucidating the complex effects of climate warming, combined with other human-driven changes of habitats on species and their interactions, requires an awareness of the possibilities and limitations of such research approaches. When developing experimental protocols to assess climate-driven mismatches, a major challenge becomes the differences between plants and pollinators in their space use (plants are sessile, pollinators are mobile).

The population dynamics of pollinators are probably controlled by resource availability such as floral resources and nesting sites at a landscape scale, whereas plants are mainly controlled by factors at the local patch scale (e.g., pollinators and/or nutrients). To overcome these scale-related restrictions, a feasible approach would be to assess potential consequences of temporal and/or spatial mismatches on plant–pollinator mutualism, if they occur. For example, a temporal mismatch may be created by warming up plants to accelerate their flowering development; “forcing” them to reach anthesis before their main pollinator is available, and thereafter track how their pollinator visitation, reproductive success, and population dynamics is affected by such a mismatch. A similar approach would be to transplant flowering plants along altitudinal gradients to simulate both flowering advancement and delay. It should be kept in mind that the effective population size, representing mate availability, will likely be reduced in such experiments causing a confounding factor that is not directly related to climate.

As many plants and pollinators are generalists (e.g., Waser et al. 1996), studying the outcome of a single interaction between two mutualistic partners exposed to a mismatch, where the partners operate on different spatial scales, may be problematic and unrealistic. We can envision one situation where manipulation would be efficient, albeit ethically questionable due to the rarity of such interactions and the involved species. In highly specialized symmetric interactions, where one plant species is pollinated by one pollinator species and where the pollinator has a restricted home range (e.g., some solitary bees), one could speculate that simultaneous experimental manipulation of both plant and pollinator densities would be achievable. Such an experiment could focus on altering the factors that are predicted to change in a warmer climate, and measure the population responses of both partners. As for many studies of biological responses to climate change, effects of temperature-mediated mismatches on plant–pollinator interactions often have to be studied through observational data (e.g., Parmesan and Yohe 2003).

Observations along natural gradients of climate conditions (e.g., Devoto et al. 2005) may be one potentially powerful tool to study effects of climate change on ecological interactions, particularly if they are combined with experiments designed to elucidate possible underlying mechanisms for the responses observed. For example, one could study how the magnitude of pollen limitation in plants (as a proxy for pollinator availability) changes along altitudinal gradients (as a proxy for climate change) (e.g., Gügerli 1998; Totland 2001). Alternatively, one could manipulate the availability of pollinators (e.g., by using cages that exclude pollinators, completely or partially, from flowers) along natural gradients of climate conditions. Such an approach could enable an assessment of how altered abundance or composition of pollinators, for example as a simulation of a mismatch, may affect plant reproduction, recruitment, and subsequent population and community dynamics under different climate conditions (see Fontaine et al. 2006 for an example).

Manipulating the availability of flowers to assess how that may impact populations of pollinators is very time and cost intensive because it requires manipulations of flower availability over large spatial scales. This might still be possible in species-poor systems with low flower abundance, or where pollinators have highly restricted

foraging ranges. Manipulation of resource availability and subsequent assessment of population dynamics of pollinators may be one way of estimating potential consequences of mismatches (see Steffan-Dewenter and Schiele 2008 for a field example). Comparing demographic processes of pollinator populations under different resource conditions, for example different types of natural or cultivated landscapes, may be an observational approach to improve knowledge on population dynamics of pollinators.

It is also important that we improve the way mismatches are quantified. Several studies of phenology use first appearance dates (both flowering and flight) as indications of mismatch. However, this variable only represents a subset of the individuals in a population, whereas the mean advancement of phenology might matter more for how climate warming affects the persistence of mutualistic interactions. Likewise, it is not necessarily the change of distributional extremes of species geographical ranges that matter for their interactions with other species, but rather the change of their optimum or mean distribution (Kelly and Goulden 2008; Lenoir et al. 2008).

19.26 Plant–Pollinator Interactions Across Time

Although previous publications have documented temporally variable pollinator environments for specific plant species (e.g., Herrera 1988) and have described intra-annual variation in plant and bee composition (e.g., Petanidou and Ellis 1993; Petanidou and Smets 1996), temporal analyses of entire plant–pollinator interaction networks are still in their infancy. This limitation is largely due to the lack of available data sets with a temporal component, which is understandable given the effort required to complete such tasks. For example, many of the earlier pollination network analyses were based on observations derived from a single season (e.g., Memmott 1999) or were aggregated across multiple seasons without regard to time scales (see references in Jordano et al. 2003). Understanding the patterns and scale of temporal variation is necessary to gauge the long-term effects of global change on plant–pollinator interaction networks. Only in the last few years have ecologists specifically addressed daily, seasonal, and annual temporal patterns in network structure (e.g., Olesen et al. 2008). In general, these studies have attempted to characterize intra- or inter-annual patterns of network structure, which is how we will summarize the findings. Here we will focus on describing empirical studies rather than studies that simulate network dynamics following species removal (e.g., Valdovinos et al. 2009; Kaiser-Bunbury et al. 2010).

19.27 Plant–Pollinator Interactions Across Space

Given that plant and pollinator taxa vary in their spatial distributions, spatial processes also likely influence their interactions (Olesen and Jordano 2002; Vázquez et al. 2009a), but only recently have spatial effects in interaction networks been addressed explicitly in empirical studies and models incorporating spatial variation. In

studies focusing on plant populations of a single species, spatial variation in the assemblages of visiting pollinators is frequently found (e.g., Eckhart 1992; Guitian et al. 1996; Fenster and Dudash 2001; Moeller 2005) and can lead to differences in pollen limitation and plant reproduction (e.g., Mustajarvi et al. 2001; Gomez et al. 2010). Additionally, landscape traits, including habitat fragmentation and quality, are known to have strong effects on pollinator communities and plant visitation (e.g., Steffan-Dewenter and Tschamtkke 1999; Klein 2009). Very little work, however, has been scaled up to ask whether community-level plant–pollinator interaction networks vary spatially, and the underlying processes influencing network-level variation across space are virtually unknown. Understanding the causes and consequences of such spatial variation is important for answering fundamental ecological and evolutionary questions of coevolution and specialization in plant–pollinator interactions as well as for applied questions of their conservation, restoration, and management. Here, we summarize and expand with some new analyses.

19.28 Climate Change and Crop Pollination

Memmot et al. (2007) simulated the effects of increasing temperatures on a highly resolved plant–pollinator network. They found that shifts in phenology reduced the floral resources available for 17 to 50 % of the pollinator species. A temporal mismatch can be detrimental to both plants and pollinators. However, the negative effects of this changed timing can be buffered by novel pollination interactions. Intensively managed monocultures usually provide floral resources for a limited time period. The survival rate and population size of the main pollinators may decrease if the foraging activity period is initiated earlier than the flowering period of the crop species. A loss of important pollinators early in the season will reduce crop pollination services later in the season. In such cases, introducing alternative food sources might be an option for farmers. In more heterogeneous agroecosystems, which are characterized by a higher diversity of crops and semi-natural habitats, pollinators may more readily survive on other crops and wild plants while waiting for their main food crop to flower.

Despite the possibility of moving crop species to areas of suitable climate, we still believe that spatial and temporal mismatches between important crop species and their pollinators are highly probable in the future. Temporal mismatches and lack of synchronicity in plant and animal phenologies are likely because crop plant phenologies probably respond to climate variables in comparable ways to wild plants. Spatial mismatches may also be likely because of the socioeconomic costs and consequences of moving food production to new areas, particularly in impoverished countries with high population density and a high degree of pollinator dependence for food production (Ashworth et al. 2009). Therefore, it is of utmost importance for global food production and human well-being that we understand the effects of climate change on animal-pollinated crops in order to counteract any negative effects.

19.29 Future Research

Temperature plays an important role in the life of plants and pollinators, and in the interactions among them. Although temperature is the factor most often studied, many other environmental factors and cues (see also the section on Evolutionary Responses to Mismatches) may also be of great influence and potentially affect species phenology, abundance, and distribution. Firm predictions of future impacts of climate warming on ecological interactions may require both knowledge of the importance of different cues to phenology and the covariance between temperature and other cues. Thus, future research focus must include not only direct temperature effects on pollination interactions, but also indirect and correlated effects of climate change. One goal should be to assess separately the direct and indirect effects of climate warming on plant–pollinator interactions. Moreover, different cues or combinations of cues may have a disproportionate influence on phenological events across species, ecosystems, and climatic regions. For example, it appears that bumble bee queen emergence in the Rocky Mountains is tied to snowmelt, as is also the case for the plants they pollinate (Inouye 2008). The activity of insects can also be strongly influenced by photoperiodicity and this effect generally increases with altitude and latitude (Bradshaw and Holzapfel 2007) potentially counteracting warming impacts. Mismatches may occur if photoperiodicity is a more important cue for insect emergence (e.g., Bradshaw and Holzapfel 2007) than for onset of flowering. Studies should simultaneously assess the importance of different cues in both plants and pollinators. In addition, we know much less about potential biological effects of climate warming on ecological interactions in the tropics than in temperate areas. One central question is whether the relative importance of different environmental cues to species' phenologies may differ across altitudinal or latitudinal gradients.

The evolutionary response to climate warming and climate-driven mismatches is of great importance for the persistence of ecological interactions. For example, it is still unclear whether genetic shifts (see the section on Evolutionary Responses to Mismatches) will be fast and strong enough to prevent species extinctions under predicted climate change scenarios (Davis et al. 2005; Parmesan 2006; Skelly et al. 2007).

Understanding the contexts in which evolution should be considered as a possible and plausible response vs. those in which it is not a likely response, remains a critical challenge for scientists studying ecological effects of climate change (Skelly et al. 2007). Future research should aim at determining the capacity of adaptive, evolutionary changes in plant and pollinator species with contrasting life-histories (Davis et al. 2005), coupled with studies on the indirect evolutionary consequences of climate warming through changes in species interactions.

19.29.1 Long-Term Studies and Simulation Approaches

Long-term monitoring programs, specifically designed to track changes in pollination services and pollination interactions in time, are rare, expensive, and time-consuming

(but see Gordo and Sanz 2005, 2006; Williams et al. 2001; Ghazoul 2005 for some examples). Ideally, such programs should involve long-term monitoring, consisting of standardized sampling (e.g., Memmott 1999; Westphal et al. 2008) of flowering plants and pollinators, and their interactions. Such sampling schemes should fulfill certain criteria. To achieve reliable data on climate-driven changes in species assemblages, or the effects of mismatches, studies need to use a high taxonomic resolution, include data on abundance and demography, and should preferably cover different climatic zones. An alternative to initiating new long-term monitoring studies would be to resample previous plant–pollinator interaction studies (e.g., Petanidou et al. 2008) and examine if observed changes may have been driven by recent climate change. It is important that such resampling procedures control for the large inter-annual variability in species abundance and link-structure that typically occur in these systems (e.g., Petanidou et al. 2008; Olesen et al. 2008).

Perhaps the most proactive research approach, which also could serve the need for rapid actions and changed conservation policies, would be to develop more advanced and realistic simulation models aimed at predicting future responses to climate change. Earlier simulation studies have shown that pollination networks may be quite resistant to perturbations (Fortuna and Bascompte 2006; Memmott et al. 2004), but that they may also experience significant structural changes when perturbed (Vázquez and Simberloff 2003; Aizen et al. 2008). Insight on the relative importance of various cues (as discussed earlier) may also be used to validate assumptions made about climate-driven shifts in phenologies or distributions in simulation models (e.g., Memmott et al. 2007; Devoto et al. 2007) or projections, such as those illustrated in Fig. 19.2. In a recent study, Deutsch et al. (2008) used empirical fitness curves related to the thermal tolerance of insects across the globe and related this to the expected geographical variation in climate warming during the next century. Interestingly, they found that tropical insects were most likely to experience deleterious effects of warming due to their narrower thermal tolerance, despite the relatively lower temperature increase expected in tropical habitats. Identification of such fitness curves of thermal tolerance for plants and their pollinators, or potential niche spaces (e.g., Schweiger et al. in press), could enable simulations of potential future mismatches among mutualistic partners. Simulations of altered nectar production under changed temperatures could also be a useful approach for a better understanding of the relationship between food availability and pollinator population growth.

19.30 Conclusions

Widespread mortality in the *A. mellifera* honeybee worldwide aptly demonstrates the fragility of this species, whose survival relies on an increasingly hostile environment. The reasons given to explain this phenomenon include pesticide use, new diseases, stress, and a combination of these factors. As a result, climate change will shift the balance between the honeybee, its plant environment, and its diseases. The honeybee has shown a great capacity to colonize widely diverse environments and

its genetic variability should enable it to adapt to such climate change. However, the fear is that climate-induced stress will, in future, compound the various factors already endangering the species in certain regions of the world. If humans modify the honeybee's environment, they also have a duty to take conservation measures to prevent the loss of this rich genetic diversity of bees. It is necessary to conduct fundamental research aimed at ascertaining the causes of mortality, as well as the effect of human-induced environmental change. Environmental impact studies in the field, as well as the use of modern genomics methods made possible by the recent sequencing of the bee genome, are expected to play a prominent role in discovering the vital stress factors for these species.

The phenology, geographic distribution, and local abundance of plants and pollinators appear to be affected by recent climate change (Figs. 19.1 and 19.2). Nevertheless, the current knowledge of the potential ecological consequences of increasing temperatures is limited and often must be deduced from indirect evidence or basic ecological knowledge of pollination interactions or studies of the mutualistic partners separately. Timing of both plant flowering and pollinator activity appears to be strongly affected by temperature, and their response appears to be linear within the limits of temperature fluctuation observed during recent decades (Fig. 19.2). Thus, plant and pollinator responses to climate warming may act in concert, although there may be considerable variation in the thermal sensitivity across species. There is also limited information on the relative importance of the factors controlling the phenology, distribution, and abundance of plants and pollinators. Temporal mismatches between plants and pollinators in early season have been documented but spatial mismatches have so far not been observed. The demographic consequences of mismatches are still little known (Fig. 19.1). Although current knowledge suggests that plant–pollinator systems may be resilient to perturbations, such as those caused by climate warming, it is premature to conclude whether increased temperatures in general will be unfavorable or positive for the persistence of many pollination interactions.

Pollination ecologists are faced with a tremendous challenge if we want to understand how future climate change might affect plant–pollinator interactions (see the section on Future Research), and reveal the importance of climate warming relative to other human modifications of natural habitats for the persistence and stability of these interactions. We believe the most important future research directions will be to monitor whether climate-driven temporal or spatial mismatches between plants and pollinators really do occur and subsequently to estimate their potential consequences for pollination interactions. The relative contribution of climate-change driven direct and indirect effects on population dynamics of species, through the effects on pollination and food availability, need to be understood in greater detail. Ultimately, this insight could enable us to understand better how community properties may change by climate-driven plant–pollinator mismatches.

Understanding the ecological and evolutionary causes and consequences of spatial and temporal variation in plant–pollinator interactions is important for both basic and applied questions in community structure and function, the evolution of floral traits, and the development of optimal conservation strategies. Phenology studies are

important in both the short-term and the long-term data they can yield. Studies like this can provide data about flowering during a single season, but sustained monitoring can yield important data associated with topics such as community change and climate change (e.g., Inouye 2008). They can also provide insights across different plant communities, allowing the comparison of different communities' responses to change.

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Chapter 20

Behavioural Defense Against Diseases and Enemies

20.1 Introduction

All living organisms are subject to infestation or attack by their natural enemies, and honeybees of the genus *Apis* are no exception. Through their long history of evolution and natural selection, they have achieved a high level of eusociality, many thousands of individual bees living together in a tightly knit social organization. Since individual bees have more than frequent contact among themselves, and since trophylaxis (the sharing and orally passing of food among members of the nest) is one of the most important and frequent aspects of the bees' social behaviour—in that it allows hormones and pheromones to be widely distributed throughout the colony—whenever a pathogenic organism is present in the colony it will be spread with great ease.

The effective defense against disease is one of the most essential achievements of the bee colony. The individual bee's immune system functions in a similar way to that of vertebrate animals, although the most effective defense mechanism that can lead to self-healing of the bee colony is the social behaviour of removing as many pathogen agents or parasites as possible from the bee colony. This behavioural defense (entrance reduction and/or stinging) prevents parasites from penetrating the bee colonies, or their killing or removal. If the dead organism is too large to remove, as with mice, the bees completely cover it with propolis. This prevents release of the pathogens during decomposition of the body. Propolis is also applied inside the brood cells before new brood is reared. Disinfection of the inside of the cell is effected by covering with secretion from the mandible and propolis. The most important defense against disease, however, is the bees' hygiene behaviour. The defense against brood diseases comprises identification and removal of affected brood. To this end the bees inspect every single brood cell. On finding an infected larva in a sealed cell, the cell capping is removed, and any sick brood is removed and finally eliminated from the colony. The beekeeper recognises defense activities against brood diseases from the scattered brood surface. If adult bees fall ill they are either forced to leave the colony or are lost during the first foraging flight. Self-healing is therefore frequently possible by increasing flight activity. This may be initiated by foraging flights or during hibernation by cleansing flights, although it is only possible if the colony is

sufficiently provided with pollen and nectar. Despite these very effective defense mechanisms, diseases, parasites and destructive insects may represent a problem for bee colonies.

Social immunity, which describes how individual behaviours of group members effectively reduce disease and parasite transmission at the colony level, is an emerging field in social insect biology (Cremer et al. 2007; Cremer and Sixt 2009; Wilson-Rich et al. 2009). This phenomenon is widespread across the social bees, ants, wasps and termites. The behaviours range from more common acts like grooming of nestmates (Rosengaus et al. 1998) and removal of dead material from the main nest area (Currie and Stuart 2001; Hart et al. 2002) to “social fever” in honeybees that is used to kill pathogens (Starks et al. 2000) and the detection and removal of pre-infectious diseased or parasitized brood (Rothenbuhler 1964; Wilson-Rich et al. 2009). Since social insects generally live in large groups of constantly interacting, related individuals, there is an increased risk of disease outbreaks and evolution of specialized parasites (Schmid-Hempel 1998). In light of this, the finding that honeybee immune pathways have a decreased number of family members or paralogs as compared to other non-social insects with complete genomes was surprising, as it indicates that honeybees may have reduced individual mechanisms of physiological defense (Evans et al. 2006). It is interesting to consider the suite of behavioural mechanisms or other traits that may have evolved at the individual and colony levels to compensate for this (Evans and Spivak 2010).

Honeybee diseases create serious problems, which must be met not only by the beekeeper but also by the bees themselves which are not usually defenseless against their enemies. *Varroa* mite is a serious pest of *Apis mellifera*, but its original host *A. cerana* (the Asian hive bee) has effective defense mechanisms. *A. cerana* also has a remarkable defense against attack by giant hornets. These hornets prey on bee colonies and are so large and well armoured that the bees are unable to sting them to death or drag them away. Instead, groups of several hundred worker bees form clusters around individual hornets attacking their hive and cook them to death by raising the internal temperature of the ball to 46 °C. This is lethal to the hornet but not to the bees. This dramatic use of heat in defense is unique. *A. mellifera*, which lives in a part of the world where giant hornets do not occur, does not have this defense.

Like other living organisms, honeybees have their diseases and enemies spectrum (Table 20.1). The spread of diseases and some enemies in honeybees is very fast because of their trophylaxis and other behaviour patterns like swarming, absconding, robbing, drifting and foraging. Some of the brood diseases of *Apis* spp. are very serious. Because of these behavioural patterns, diseases can spread from contiguous areas of one country to other countries. Above all, tropical Asia is believed to be the evolutionary homeland of *Apis* spp. and disease and enemy spectrum is likely to be greater. Honeybees are attacked both at brood and at adult stages by micro-organisms. Therefore, the diseases of honeybees are divided into brood diseases and adult diseases. Brood diseases are of serious concern to beekeeping professionals. Many of the diseases, occurring in honeybee colonies do not show apparent symptoms of infection for long period and cause catastrophic destruction of honeybee colonies in the apiaries whenever they appear.

Table 20.1 Pests and diseases of *A. cerana*

Name of pests/diseases	Country	Honeybee species	Reference
American foul brood	Thailand	<i>A. mellifera</i>	Akratanakul (1984)
	Burma	<i>A. mellifera</i>	Zmarlicki (1984)
	India	<i>A. cerana</i>	Sekariah and Seth (1959)
	Australia	<i>A. mellifera</i>	Bailey (1985); Rhodes and Goebe (1986)
European foul brood	New zealand	<i>A. mellifera</i>	Winter (1980)
	Thailand	<i>A. mellifera</i>	Akratanakul (1984)
	India	<i>A. mellifera</i>	Bailey (1974); Kshirsagar and Godbole (1974)
	India	<i>A. cerana</i>	Bailey (1974); Kshirsagar and Godbole (1974); Thakur (1976)
	India	<i>A. mellifera</i> , <i>A. cerana</i>	Abrol and Ball (2006); Abrol (2008b)
	Australia	<i>A. mellifera</i>	Bailey (1985); Rhodes and Goebel (1986)
Sacbrood	India	<i>A. cerana</i>	Kshirsagar et al. (1981a, 1982)
	Pakistan	<i>A. mellifera</i>	Rafiq (1984)
Thai Sacbrood	Australia	<i>A. mellifera</i>	Rhodes and Goebel (1986)
	Thailand	<i>A. cerana</i>	Bailey (1982)
	India	<i>A. cerana</i>	Bailey (1982); Abrol and Bhat (1990); Abrol (1993a, b)
	Nepal	<i>A. cerana</i>	Bailey (1982)
Kashmir bee virus disease	India	<i>A. cerana</i>	Bailey (1982)
	Australia	<i>A. mellifera</i>	Rhodes and Goebel (1986)
	India	<i>A. cerana</i>	Bailey et al. (1976); Bailey (1982); Kshirsagar et al. (1975); Shah and Shah (1991); Shah and Shah (1976; 1979)
Bee paralysis	Hongkong	?	Gochnauer (1978)
	Australia	<i>A. mellifera</i>	Bailey (1976); Gochnauer (1978)
Acarine disease	Newzealand	<i>A. mellifera</i>	Winter (1980)
	India	<i>A. cerana</i>	Dhallwal et al. (1974); Adalakha (1976); Thakur (1976)
Endoparasitic mite <i>Acarapis woodi</i> Rennie	India	<i>A. cerana</i>	Singh (1957)

Table 20.1 (continued)

Name of pests/diseases	Country	Honeybee species	Reference
Ectoparasitic mites	Japan	?	Delfinado (1963)
Ectoparasitic mite, <i>Varroa jacobsoni</i>	India	<i>A. cerana</i>	Abrol (1993c, 1996b, c)
Varroa destructor	India	<i>A. cerana</i> , <i>A. mellifera</i>	Abrol et al. (2006); Abrol and Sharma (2007), 2009)
Tropilaelaps clareae	India	<i>A. cerana</i> , <i>A. mellifera</i>	Abrol (1995, 1996a)
Tropilaelaelaps koenigerum	India	<i>A. cerana</i>	Abrol and Putatunda (1995)
Tyrophagus longior	India	<i>A. cerana</i> , <i>A. mellifera</i>	Abrol et al. (1994a, b)
European foul brood	India	<i>A. mellifera</i> , <i>A. cerana</i>	Abrol and Ball (2006); Abrol (2008a, b)
Nosema disease	India	<i>A. cerana</i>	Singh (1975); Thakur (1976); Kshirsagar (1982)
	Bangladesh	<i>A. cerana</i>	Nessa et al. (1983)
	Australia	<i>A. mellifera</i>	Bailey (1985)
	India	<i>A. mellifera</i> , <i>A. cerana</i>	Abrol (2008a, b)
Neocyphophthalmus mite	India	<i>A. cerana</i>	Kshirsagar (1968)
	Pakistan	<i>A. cerana</i>	Delfinado-Baker and Baker (1983)
	Nepal	<i>A. cerana</i>	Baker and Delfinado (1976)
Wax moths	India		
Galleria mellonella	India	<i>A. cerana</i> , <i>A. mellifera</i> , <i>A. dorsata</i> , <i>A. florea</i>	Abrol and Kakroo (1996)
Achroia grisella	India	<i>A. cerana</i> , <i>A. florea</i>	Abrol and Kakroo (1996)
Pseudoscorpion	India	<i>A. cerana</i>	Abrol and Kakroo (2003)
Ellingsenioides indicus	India	<i>A. cerana</i>	Abrol and Kakroo (2003)
Hawk moths	India	<i>A. cerana</i> , <i>A. mellifera</i>	Abrol and Kakroo (2003)
Acherontia styx	India		
Wasps			
Golden wasp <i>Vespa orientalis</i>	India	<i>A. cerana</i>	William (1988)
The giant wasp <i>Vespa velutina</i>	India	<i>A. cerana</i>	William (1988); Shah and Shah (1991)

Table 20.1 (continued)

Name of pests/diseases	Country	Honeybee species	Reference
The giant wasp <i>Vespa magnifica</i>	India	<i>A. cerana</i>	William (1988); Abrol (2006)
The Yellow banded wasp <i>Vespa cincta</i>	India	<i>A. cerana</i>	William (1988)
<i>Vespa manadrina</i> , <i>V. tropica</i> , <i>V. ducalis</i> , <i>V. analis</i> , <i>V. asiatica</i> , <i>V. austriaca</i> , <i>V. nursei</i> <i>V.flaviceps</i> , <i>V. structor</i> ; <i>V. germanica</i> , <i>V. auraria</i> , <i>V. basalis</i> , The Yellow wasp <i>Polistes hebraeus</i>	India	<i>A. cerana</i>	William (1988); Abrol (1994a)
Bee eater birds			
Green bee eater <i>Meropes orientalis</i> , <i>Meropes apiaster</i> , <i>M. superciliosus</i>	India	<i>A. cerana</i>	Abrol (1994b)

20.2 Behavioural Defense

Honeybees have evolved behavioural traits during their evolutionary history to reduce threats from invading pathogens. In open nesting, the honeybees are exposed to environmental stresses, such as rain, wind and temperature changes, and need to defend the entire nest surface against predators. When facing an enemy, the bees are found to retreat or to attack on the enemies and the attack or retreat strategy is highly dependent on nest structures and placements. The open nesting honeybees (*A. florea* and *A. dorsata*) have some kind of adaptation to survive the open environment. The rock bees (*A. dorsata*) prefer inaccessible, but often conspicuous, nesting sites in high trees or cliffs and also the rock bees defend its nests aggressively when they are disturbed. In little bee (*A. florea*) colonies defend themselves against visually hunting predators by building low, dispersed, hidden nests, and use sticky barriers to protect them from ants. In Indian bee (*A. cerana*), the colonies rely upon nesting inside cavities for protection against large predators, and on direct attacks by their relatively large workers to repel smaller enemies such as ants. These offer strong protection by restricting accessibility to the nest and at the same time constitute a limited and valuable resource in them, to be actively defended if in danger. Nests of open-nesting species, *A. florea* and *A. dorsata*, are protected by a three to six layer curtain of bees over the comb. *A. cerana* colonies lack these curtains but are protected by their nest cavity walls. Guard bees recognise members of their own colony by a hive odour specific to each colony. Having the same odour, returning foragers have no difficulty in passing through the hive entrance, but most foreign intruders, including worker bees from other colonies with a different odour, are repelled by the defending guards. Defense is an important behaviour required for the survival of the colony, since colonies contain attractive resources, such as brood and honey for various predators. Different innate and behavioural mechanisms which protect honeybees from various diseases and enemies are as given below:

20.3 Immunity

Honeybee colonies are infected by numerous pathogens, including bacteria, fungi, viruses, and parasites; for example, American foulbrood (AFB), caused by Gram-positive bacterium *Paenibacillus larvae* (Genersch et al. 2006), is one of the most destructive diseases affecting honeybee larvae. AFB is highly infectious and capable of killing honeybee colonies. In addition, the ability to form spores allows *P. larvae* to remain viable for many years and to survive under adverse environmental conditions (Matheson and Reid 1992). AFB leads to a decrease of the honeybee population and honey production, causing serious economic losses and problems for the beekeeping industry (Ellis and Munn 2005; Genersch 2010). With the honeybee population declining in many parts of the world (van Engelsdorp et al. 2008), multiple factors, such as pathogens and other stressors, are likely to be involved in the current colony depopulation. There has been growing interest in the immune defense mechanisms

of honeybees concerning the risk of infection by pathogens. To combat invading pathogenic micro-organisms, honeybees possess effective innate immune systems. Antimicrobial peptides are synthesized and secreted in response to bacterial infection. Several antimicrobial peptides, with a broad spectrum of antibiotic activities against a variety of bacteria, have been reported in European honeybee, *A. mellifera*. Transcript levels of antimicrobial peptides genes, abaecin and defensin, against *P. larvae* infection have been studied (Evans 2004). The native honeybee of Japan, *A. cerana japonica*, is widely distributed in Japan. It is characterised by its dark color, small size and gentle behaviour. Due to its tolerance to pathogenic micro-organisms and parasitic mites, Yoshiyama and Kimura (2010) reported that like social insects the *A. c. japonica* honeybees have evolved behavioural traits during their evolutionary history to reduce threats from invading pathogens. To gain molecular insight into AFB tolerance, antimicrobial peptide cDNA clones, abaecin and defensin, were isolated from *A. c. japonica*. Genomic analysis revealed that honeybees, *A. mellifera*, carry fewer known immune genes than *Drosophila melanogaster* or *Anopheles gambiae*, suggesting that a compensatory system to secure diseases tolerance exists (Honeybee Genome Sequencing Consortium 2006). Honeybees have developed several kinds of behaviour in order to avoid and eliminate invading pathogens (Cremer et al. 2007). In particular, Eastern honeybees are known to have established defense behaviours, such as heat balling, and social waves against hornet attack (Ono et al. 1995; Kastberger et al. 2008). In addition, *A. cerana* spp. have developed specific defense behaviours that protect the colony by preventing infectious diseases and parasites, such as AFB and *Varroa* mites. *P. larvae*-inoculated larvae were removed by adult worker bees before the capped stage (Chen et al. 2000). *A. cerana* worker bees are able to remove *Varroa* mites from their bodies and nest mates by grooming. *A. cerana* are able to recognize capped brood cells infected by *Varroa* mites, and can open cells and kill mites by biting (Peng 1987b). Thus, hygienic behaviours could contribute to decreasing the potential risks of spreading viral diseases transmitted by *Varroa* mites. To speculate the relationship between the innate immune system and social defense behaviour in Japanese honeybees, further research is needed to determine the function of other immune effector molecules against *P. larvae*.

Gliński and Jarosz (2001) reviewed the information on immunity of honeybees to various diseases. The honeybee is subject during its life to a nearly continual challenge by different saprophytic and pathogenic micro-organisms (bacteria, viruses), protozoan and metazoan parasites (larvae of *Senotainia tricuspis*, *Mermis* sp.) and parasitic mites (*Acarapis woodi*, *Varroa jacobsoni*). In response to infection and injury, a variety of immune processes have evolved to suppress invaders that have succeeded in reaching the haemocoel (Dunn 1986). The anatomical and physiological barriers formed by the cuticle, midgut and tracheal system play a crucial role in protecting the insect against the penetration of microbial intruders into the haemolymph (Gliński and Jarosz 1995a). When the outer protective barriers are broken, the invader encounters the internal immune responses active in the coelomic cavity of the bee (Gliński and Jarosz 1995b, c).

Haemocytes, the major cellular components in the immune system are involved in the cell-mediated immune reactions (Salt 1970) while humoral defense is attributed to the activity of soluble protective factors, both innate and inducible of

insect haemolymph (Boman and Hultmark 1987). The ability to discriminate between self and non-self is the first prerequisite that generate the immune defense phenomena (Ratcliffe and Götz 1990). The recognition and attachment events that initiate cellular defense response in innate system are regulated by humoral and by membrane-bound molecules that discriminate self versus non-self. Bacterial LPS or peptidoglycan molecules and mannose, galactose or glucan residues in the case of fungi, hemolin and lectins are the pattern-recognition molecules (Schmidt et al. 1993). In addition, the honeybee as a social insect has developed mechanisms that efficiently protect both the individual and the entire colony against a great number of microbial pathogens (Gliński and Jarosz 1994). The environmental polluting agents and pesticides can undoubtedly impair the non-self response system and protective defense of the bee against pathogens, parasites and predators.

20.3.1 Antiviral Protection

Till date, more than 15 distinct viruses have been characterized from honeybees. Most of them persist as inapparent infections, however, the latent viruses can be induced to multiply to readily detectable levels under stress conditions. All known viral diseases of honeybees are specific for only one stage in the life cycle: acute paralysis virus (APV), chronic paralysis virus (CPV), cloudy wing virus (CWV) attack adults whereas sacbrood virus (SBV) and black queen cell virus (BQCV) are restricted to larval stages (Allen and Ball 1996). The most common route of infection with bee viruses is the ingestion of intact virions by feeding insects. The ingested viral particles may infect the epithelial cells of the midgut or pass through the gut wall to enter susceptible target cells in the insect body. Bee viruses are polytrophic because they infect most, if not all, tissues of the host. The most important role in virus–host relation is played by a specificity of the virus to the insect, the portal of entry for virions to the host body and the efficacy of the bee defense mechanisms. The bee viruses, similar to other insect viruses, have evolved mechanisms for avoidance or depression of the insect host responses. These mechanisms allow them to survive for long periods in their hosts and cause latent infections (Ratcliffe et al. 1985).

A number of the non-immunological factors modify and control virus replication and the outcome of viral infections. Commonly known protecting the bee against viruses are the thresholds present in the midgut and formed by the biochemical environment of the gut juice, the peritrophic membrane and the midgut epithelium. In most cases, the peritrophic membrane limits the spread of infection because of its impermeable or hardly permeable barrier to viral particles (Smith et al. 1993). Only viruses that passed the peritrophic membrane may infect the gut epithelium. They could trespass the epithelial lining of the midgut, infect coelomic cavity, cause a viraemia and then infect body tissues. A prerequisite for infection is adsorption of virions to the specific receptors on the surface of susceptible cell. One cause of inhibition of viral replication is the production of interferons released from virus infected cells within a few hours after viral invasion. Insect interferons are glycoproteins (20–34 kDa) stable to heat and to extremes of pH.

It can be hypothesized that in bees, like in mammals, interferon could act by entering infected cells and depressing their DNA, so as to produce translation inhibitory protein. This protein can, in turn, block the takeover of cell ribosomes by viral RNA, and hence inhibits virus replication. The another factor that controls viral infections is lack of specific receptors at the plasma membrane to bind of ligands. The binding of ligands to receptors stimulates cells to change shape, endocytose, proliferation or modulation of cell functions. Once a viral nucleoprotein penetrates the plasma or vesicular membrane, it must make its way to the site in the cell where it will replicate. Within the infected cell, replication of bee viruses occurs entirely within the cytoplasm. Serologically related viruses, sometimes by unrelated or defective viruses may block the viral receptors on the susceptible cells. The blocking of viral receptors on sensitive cells is an effective mechanism of antiviral defense.

Viral infections induce unspecific cell defense reactions such as phagocytosis and nodule formation. Phagocytosis of virions develops in early and late stages of infection when viruses are liberated into the haemolymph from damaged cells. Phagocytosis and nodule formation is not always effective in prevention and control of viral diseases. The final result is both the recovery of the bee host due to the efficacy of the defense mechanisms or the viral invader prevails and the host is killed. A small number of viruses or viruses of a low virulence infecting the insect are killed by haemocytes whereas heavy infections or virulent strains can replicate and kill specific types of haemocytes involved in antiviral defensive reactions. Finally, many microbial parasites have evolved mechanisms for avoidance or depression of the insect host response. These mechanisms allow parasites to survive for long periods in the bee organism, which act as a source of new infection within a bee colony. Obviously, the neurons in the thoracic and abdominal ganglia protect replicating bee paralysis viruses from phagocytosis and other haemocytic defense reactions.

20.3.2 *Bacterial Infections and Immunity*

Bacteria associated with bees are widely distributed in soil, water, and air, stored bee food, surface of plants and skin of other living creatures. In most infections, bacteria invade the bee body cavity through the intestines with ingested bacteria contaminated food. Bacteria may also infect the bee body via spiracles or with the aid of the piercing or chewing mouthparts of predators and external parasites; for example, via cuticular abrasions caused by a stenophagous mite *Varroa jacobsoni* (Gliński and Jarosz 1992). Any break of the cuticle or the alimentary canal is a portal of entry for bacterial invaders. The honeybee larvae can develop the American foulbrood (*Paenibacillus larvae larvae*), European foulbrood (*Mellissococcus pluton*) and the powdery scale disease (*Bacillus pulvifaciens*) whereas in adult bees septicaemias result mostly from *Pseudomonas aeruginosa*, *Hafnia alvei* and *Enterococcus faecalis* infections. Saprophytic and plant pathogenic bacteria may also accidentally induce the fatal septicaemia after invading the haemocoel.

Bee body coverings and the biochemical environment of the midgut juice by bacteriostatic or bactericidal action effectively restrict the development of most infections caused by bacterial saprophytes (Jarosz 1993, 1995). If the bacteria succeed in reaching the haemocoel through mechanically injured or enzymatically damaged anatomical protective barriers, they generate the internal immune responses in body cavity. The bee has an open circulatory system and numerous haemocytes are contained in its haemolymph. Prohaemocytes, plasmatocytes, granular cells, cystocytes, sphaerula cells and encytops are the cells that comprise the bee haemocyte population (Gupta 1991). Commonly known haemocyte-mediated defense reactions of bees consist of phagocytosis, encapsulation, cytotoxicity, secretion of materials to damage foreign organisms (humoral encapsulation) or to modulate immunocyte functions. Haemocytes are able to recognize self and non-self. At least three possible factors may act as non-self-recognition molecules in bees, that is the components of the prophenoloxidase cascade, lectins and chemokines. The prophenoloxidase activating system (proPO) consists of a cascade of enzymes and associated factors such as various plasma receptors, serine proteases and inhibitors (Söderhäll and Smith 1986). The multifunctional proPO cascade is comparable to the vertebrate complement system in a number of ways. Both systems generate opsonic intermediates that assist in the initial recognition and attachment phases of phagocytosis.

Much interest has recently been generated by the observation that the pPO is involved in immune recognition and cellular communication. This system itself can react to foreign materials and be converted into its active form by microbial products. The granular cell type may contain not only lectins but also components of the proPO system. This cell type rapidly degranulates in contact with non-self materials to release the non-self-recognition molecules. The occurrence of lectins in haemolymph of bees is scarcely documented. They have a dual function in insects, in defense and in development. The lectins enhance recognition and phagocytosis by insect haemocytes and they have ascribed roles in nutrition and development. They probably are involved in tissue reorganization and cell adhesion (Olafsen 1986). Hemokines, a group of small secreted proteins, function as important regulators of the immune system by inducing haemocytes to migration and by modulation haemocyte–antism interactions (Chadwick and Aston 1991). The haemocytes can engulf and destroy smaller foreign objects such as bacteria or fungal spores, but larger parasites, bacterial clumps or fungal hyphae, are encapsulated by several haemocytes and then removed from circulation.

The cellular immune responses have been shown to be accompanied by changes both in the number of circulating haemocytes and the relative proportions of different haemocyte types in the blood. Generally, the infection mobilizes sessile haemocytes to migrate and increases a percentage of round plasmatocytes (Bahadur 1993). Predominant cells involved in phagocytosis are plasmatocytes, followed by granular cells (Sharma et al. 1986). Although the cells involved in the process of phagocytosis in the bee are well recognized, the mechanisms or the factors involved in this process and the role they play are not yet clearly understood. The engulfed bacteria are digested in phagolysosome by released hydrolytic enzymes, primarily lysozyme, alkaline phosphatase, ribonucleases and phospholipases.

Relatively little is known about oxygen-dependent microbial killing mechanism in insects. Superoxide anions have been detected in several lepidopterans and there is evidence that lipophorin is involved in production of these molecules. (Arakawa et al. 1996). Nitric oxide synthetase (NOS) has been detected in insect fat body and Malpighian tubules. The fat body NOS is induced by bacterial LPS. The generated nitric acid acts as signaling/messenger molecule or as a toxic anti-microbial agent (Mims et al. 1995). The role of factors such as proPO, melanins and recruitment factors cannot be excluded in phagocytic process. They all may increase the number of collisions between phagocytes and foreign bodies and mobilize sessile haemocytes. In some instances, however, the captured bacteria may even multiply within the phagocytes, causing their death. Released bacteria then multiply in the insect blood and the bee perishes due to a fatal septicaemia.

Cellular encapsulation of large foreign invaders is a common phenomenon of cell-mediated immune reaction protecting the insect host. Components of the proPo system could function as signaling molecules to promote encapsulation. A 400 kDa complex consisting in part of prophenoloxidase, phenoloxidase, and an interleukin 1 was isolated from *Manduca sexta* haemolymph (Beck et al. 1996). The invader is enclosed in several layers of cells and the capsule-like so formed melanizes and strictly isolates the parasite from circulation. The primary stimulus initiating the encapsulation may originate from either foreign substance present on the surface of an invader or metabolites and waste products released by the parasite. The granular cells that contact and recognize the foreign body as a non-self structure degenerate to release sticky proteins that attract the plasmatocytes. Melanization is not a regular process of cellular encapsulation. Only living organisms induce both encapsulation and melanization. The melanization of the capsule depends on the proPO activating system that kills the microorganisms and parasites, isolating them from the rest of the body in the hard and impermeable capsule. Death of larvae and embryos of parasites, adolescent protozoans and nematodes may result from asphyxiation or the accumulation of toxic wastes in the capsule. Encapsulated parasites may die through the toxic effects of quinones that contribute to melanization (Vey and Götz 1975).

Nodule formation is a phenomenon in response to both animate and inanimate substances that cannot be removed from circulation by phagocytosis. In this cellular reaction, the haemocytes loaded with bacteria are entrapped by a coagulum that is produced by the degranulating granular cells and then centrally melanized. A sheet of blood cells surrounds the entrapped invaders in that coagulum in the centre. The fate of nodulated bacteria depends on their virulence. In general, bacterial saprophytes are quickly killed within melanized nodules while pathogenic bacteria may subsequently liberate from the nodules into the haemolymph (Ratcliffe and Rowley 1979).

Cell-free immune reactions involve synthesis and release of several antibacterial immune proteins, some capable of killing both Gram negative and Gram positive bacteria. The expression of this multicomponent humoral immune system requires the de novo synthesis of a specific immune mRNA in the fat body and response peptides and small protein synthesis with broad antibacterial activity (Boman and Hultmark 1987; Jarosz 1979). The bee responds to fungal infections by altering immunocompetent

Fig. 20.1 Sequences for the precursor of apidaecins Ia and Ib and amino acid sequence of apidaecins Ia, Ib, III and III of the honeybee, *A. mellifera*. (Gliński and Jarosz 2001)

Proapidaecin Ia/b EAKPEAKP	GNNRPVYIPQPRPPHPR I/L
Apidaecin Ia	GNNRPVYIPQPRPPHPRI
Apidaecin Ib	GNNRPVYIPQPRPPHPRL
Apidaecin II	GNNRPIYIPQPRPPHPRL
Apidaecin III	GNNRPVYISQPRPPHPRI

E Glutamic acid	G Glycine	I Isoleucine
A Alanine	N Asparagine	Q Glutamine
K Lysine	R Arginine	H Histidine
P Proline	V Valine	Y Tyrosine
L Leucine		

cell motility. Haemocytes have been shown to migrate towards fungal spores and hyphae. Such migratory responses may help to explain the selective depletion of insect cells from circulation that is often evident during cell-mediated immune reactions directed against parasitic fungus. Among the great majority of immune mediators in insects which have been infected with bacteria, lysozyme, apidaecins, abaecin and hymenoptaecin have been found to exist in the honeybee (Gliński and Jarosz 1995c). The bee lysozyme is a relatively small molecule (about 15 kDa), representing a group of true lysozymes shared characteristics with the chicken type lysozyme. The concentration of lysozyme in larval honeybees and in adults ranges from 5.0 to 25.0 µg/ml, and in pupae from 5.0–10.0 µg/ml of haemolymph. Bacterial infection or artificial inoculation of bacterial saprophytes increase the concentration of lysozyme in haemolymph of bee larvae even to more than 1,300 µg/ml whereas in flying worker bees to not more than 40.0 µg/ml. It is hypothesized that in brood lacking of the inducible apidaecin-family antibacterial response peptides the enhanced potency of lysozyme reduced the risk of infection of pre-imaginal stages by saprophytic bacteria (Gliński and Jarosz 1993). The peptidoglycan fragments of Gram positive bacteria released by the lytic action of lysozyme act as a very potent elicitors of antibacterial peptides such as apidaecins. In several cases, lysozyme acts as a synergistic with the smaller cationic peptides (Bang et al. 1997). The apidaecins represent a family of inducible, non-helical, small (about 2 kDa) proline-rich antibacterial peptides of activity mostly against Gram-negative bacteria (Casteels et al. 1989). They are the most prominent components of the honeybee inducible cell-free defense against bacterial invaders. Apidaecins of the honeybee consist of four closely-related peptides (Ia, Ib, II and III) composed of 18 amino acid residues each (Fig. 20.1). Biologically active isoforms appear in haemolymph of the adult bee, whereas the inactive precursor molecules—proapidaecins—exist in the blood of the last instar larvae. Conversion of the apidaecin precursors into active peptides could occur through a stepwise cleavage of dipeptides ending either a proline or an alanine.

Table 20.2 Antibacterial activity of apidaecin Ia, Ib and II *A. mellifera* (Casteels et al. 1989)

Bacterial strains	Minimum inhibitory concentration ($\mu\text{g/ml}$)		
	Ia	Ib	II
<i>Agrobacterium tumefaciens</i> DSM 3129	0.2	0.2	0.2
<i>Erwinia salicis</i> NCPPB 2530	0.02	0.02	0.02
<i>Escherichia coli</i> NCTC 9001	0.1	0.1	0.2
<i>Pseudomonas syringae</i> pv. tomato NCPPB 1106	0.2	0.1	0.1
<i>Rhizobium meliloti</i> ZB 314	0.1	0.02	0.02
<i>Salmonella</i> Newport CD 94	0.2	0.2	0.2
<i>Salmonella typhimurium</i> ATCC 23565	0.1	0.1	0.1
<i>Serratia marcescens</i> ATCC 17991	200	200	200
<i>Shigella flexneri</i> CP 87	0.1	0.1	0.1
<i>Corynebacterium insidiosum</i> NCPPB 1109	50	50	100
<i>Bacillus alvei</i> LMG 6922	200	200	200
<i>Bacillus megaterium</i> QMB 1551	150	100	100
<i>Bacillus subtilis</i> NRRL-B-237	200	200	200
<i>Bacillus thuringiensis tenebrionis</i> DSM 2803	200	200	200

Structural analysis suggests that apidaecins contain both a constant region responsible for potency and a variable region which dictates the antibacterial spectrum (Casteels-Josson et. al. 1993b).

A sharp increase of apidaecin transcript levels occurs 4–6 h after infection, followed by a steady rise of several more hours. Peak concentration in the haemolymph is within 36 h post-infection and then the concentration of the apidaecin compounds declines in bee blood gradually for the next 3–4 days. It is now known that the mode of action of the apidaecins is to attack bacterial cell membrane by the ionophoretic action, they form voltage-dependent ion channels causing leakage and cell death. This activity is non-specific and so apidaecins have a fairly broad range of activity. They are highly active against Gram-negative bacteria, but show only a weak activity against Gram positive bacteria. The spectra of activity for all isoforms of apidaecins are very similar, and seems to be bacteriostatic rather than bactericidal. The activity of apidaecins is directed against bacteria present commonly in the bee environment (Table 20.2).

It can be suggested that the marked activity of apidaecins directed against enteric bacteria, plant-associated and phytopathogenic bacteria developed in the bee as a defense mechanism active against the bacterial species contaminating plant and

Apis YVPLPNVPQPGRRPFPTFPQGPFNPKIKWPQGY

Fig. 20.2 Complete amino acid sequence of abaecin in the honeybee, *A. mellifera*

water sources visited by flying worker bees. The presence of inactive precursor of apidaecins in the haemolymph of brood may reflect differences in the immune status of the larval and adult stages of the honeybee. The risk of brood infection by bacteria contaminating the worker bees is reduced by factors of antibacterial activity of royal jelly, honey and pollen. Larva responds to bacterial infection by increasing the level of lysozyme in the haemolymph (Gliński and Jarosz 1995b).

A second antibacterial protein that was originally discovered from the honeybee is abaecin (Casteels et al. 1990). It is a large inducible, proline-rich, helical peptide (about 4.0 kDa) of antibacterial activity. Unlike the apidaecins, the range of activity of abaecin for bacteria is quite narrow and confined to a limited number of both Gram negative and Gram positive bacteria. The molecule of abaecin contains 10 proline residues but not cysteine (Fig. 20.2). Abaecin reveals striking similarities to apidaecins in *A. mellifera* and dipterocins in flies. Matching patterns of prolines and hydrophobic amino acids are present in the first 18 residues of each of the three immune peptides.

Although the abaecin is induced upon microbial infection of the bee body cavity, its contribution to antibacterial defense of the bee remains still unclear. The mode of action of the abaecin is to alter the permeability of the outer membrane of the target bacterium. It is thought that the result of this action by abaecin allows improved access to the cell wall for lysozyme and to the cytoplasmic membrane for the apidaecins. Most probably abaecin, like apidaecins, represents an advanced level of internal cell-free antibacterial immunity of the bee. They both represent the highest level of adaptation of *A. mellifera* response system to destroy plant pathogenic and enteric bacteria present ubiquitously in the bee living niche. In the clarifying the honeybee haemolymph from bacteria participates hymenoptaecin (Casteels et al. 1993), apart from apidaecin-family peptides and abaecin. Hymenoptaecin is a glycine-rich peptide having 93 amino acid residues (about 10 kDa) with bactericidal activity for Gram negative and Gram positive bacteria. This inducible peptide requires higher doses of bacteria for induction and appears in the haemolymph at later time post-infection and at lower concentration than apidaecins.

20.3.3 Immune Defenses Against Pathogenic Fungi

The best known protective mechanisms active in the honeybee to fungal infection, notably in chalkbrood and stonebrood, are in the anatomical structure of the bee body (Barr and Shope 1975; Orihel 1975). The impermeable and hard cuticle, the biochemical environment of the midgut juice, peritrophic membrane of the midgut and tracheal system form together a mechanical and physiological barrier effectively protecting the bee's body cavity against fungal invasion. A relatively low humidity in the tracheae is an important factor in restricting germination of spores and growth of

fungus in the bee respiratory tract. Waxes and unsaturated fatty acids impregnating the cuticle or present on its surface have a potent antifungal action. Competition for food between gut bacteria and fungi could efficiently eliminate massive doses of fungal spores from the gut. Nevertheless, chitinase-producing moulds and yeasts can actively penetrate the cuticular lining of the body and then infect the haemocoel. The cuticle damaged mechanically or enzymatically by growing hyphae also allow bacteria to enter body cavity and develop fatal septicaemias.

Non-degradable fungal materials are encapsulated by a large mass of haemocytes that serve as a barrier between the haemocoel and the object. Bee haemocytes may also directly kill fungal spores and other small foreign molecules in phagocytic process (Götz 1986). These cellular immune reactions have been shown to be accompanied by changes both in the number of circulating haemocytes and in the relative proportions of different haemocyte types in the blood (Hink 1970). The infection of the haemocoel initiates a premature differentiation of haemocytes and their migration towards chemotactic stimulus. Phagocytosis predominates when the body cavity is exposed to small number of bacteria or fungal spores.

Encapsulation is the most effective haemocyte-mediated immune response in protection of insect haemocoel in fungal infections. This cell-mediated reaction consists of the formation of a capsule-like envelope around foreign objects with a diameter more than 10 μ m that cannot be phagocytized by a single cell. The capsule is formed by attaching blood cells, mainly granular cells and plasmatocytes. The granulocytes release chemotactic factors which attract the plasmatocytes to form the outer layer of the capsule around the encapsulated fungus. The role of the phenoloxidase activating system cannot be excluded in phagocytosis and melanization of encapsulated insect pathogenic fungi.

Neither lysozyme nor the haemolymph response proteins seem to inhibit or destroy spores or fungal mycelia in the invaded bee. The honeybee generate several groups of humoral immune factors to resist bacterial infections. The apidaecin-family peptides, abaecin and hymenoptaecin, the most prominent components of the honeybee inducible humoral defense, are inactive entirely to fungal invaders.

20.3.4 Protective Mechanisms in Protozoan Invasions

The protozoans attack the epithelial cells of the midgut (*Nosema apis*, *Leidyana apis*) or Malpighian tubules (*Malpighamoeba mellificae*) of the adults only (de Graaf et al. 1993, 1994). Pathological changes in epithelial cells and derangement of digestive processes by *Nosema apis*, both of which led to malnutrition and premature death of the invaded bees. In the parasitized individuals, desquamation of pathologically altered epithelial cells loaded with parasites, and their removal with feces, lowers the possibility of massive invasion of other parts of gut epithelium. Scars that develop at the site of removed dead epithelial cells, and newly developing epithelium, prevent the migration of the bee microflora from the gut into the body cavity. Variations in resistance could be assigned to the activity of chymozin in the honeybee ventriculus since this enzyme by improving the development of the peritrophic membrane

prevents *Nosema apis* spores for coming into close contact with the epithelial gut cells.

When spores invade the bee body cavity, cell-mediated defense reactions seem to play a crucial role in the control of *Nosema apis* invasion. Very often, haemocytes aggregate around the parasite and are active in phagocytosis and nodule formation. Cysts and spores of parasites, like vegetative forms, can be effectively encapsulated. The effectiveness of haemocytic reactions in protozoan invasions is high but the parasites become increasingly difficult to eliminate as invasion progresses. Bacterial infections associated with the mechanical destruction of host cells by the developing protozoan accelerate the death of bees.

The protozoan *Malpighamoeba mellificae* attacks the epithelium of the Malpighian tubules, which usually become swollen and degenerated. The greatly distended tubules occasionally rupture and elicit a massive inflammatory response. Since the number of parasitic cysts produced in the body of the bee is low, massive lethal invasions of bees are not frequent. The anatomical protective barriers of the gut limit the destructive action of the parasite.

20.4 Mechanisms of Resistance to the Mite *Varroa jacobsoni*

Of about one hundred species of mites which live in or around honeybee colonies in various parts of the world, three mite species are dangerous to the honeybee: *Varroa jacobsoni*, *Acarapis woodi* and *Tropilaelaps clareae*. The pathogenic effects of *V. jacobsoni* invasion closely related to the number of parasites and their developmental stage are attributable to the mechanical injuries, depletion of haemolymph proteins and to toxic effects of the parasite. Furthermore, the parasitic mite, induces latent viral infections (Allen and Ball 1996), and transmits bacterial and fungal infections to the recipient bee host (Gliński and Jarosz 1992). It is possible that the parasite impairs the internal defense reactions of the bee and brood (Gliński and Jarosz 1984, 1988a). At least, the five mechanisms that minimize the impact of *V. jacobsoni* on honeybees have so far been discovered: grooming behaviour, removal of mite infested brood, rapid development time, unattractive brood for the mite and infertility of mites on some bees brood (Boecking 1994). In the Asian bee *A. cerana* exists behavioural tolerance to *V. jacobsoni*. *A. cerana* grooms herself, removing the mite, while the European bee does not or at most grooms very little. After *A. cerana* caught a mite, it would kill it by biting and crushing it, then taking it out of the hive. Moreover, successful reproduction of *V. jacobsoni* on *A. cerana* is limited by seasonal occurring drone brood and is lacking in worker brood (Rath 1991; Rosenkranz et al. 1993). The infested drones become weakened by the parasitic mites, are not able to uncapping their cells, and in consequence they die together with mites inside uncapped cells. The bees which show the hygienic behaviour uncap and remove bee pupae that contain a Varroa mite. Behavioural tolerance of *A. mellifera* to *V. jacobsoni* was found in Africanized Honeybees (AHBs) and European Honeybees (EHBs) from Brazil, Tunisia and Uruguay (Ritter et al. 1990). In Varroa-tolerant *A. mellifera* colonies there is a reduced fertility of *V. jacobsoni* in worker brood compared to the successful reproduction of the mite in drone brood.

One of the reasons why *V. jacobsoni* is not a problem with the Asian bee is the short period of time that the pupae is capped, which does not permit a long enough time for the mite to develop. Also differences in the susceptibility to *V. jacobsoni* in *A. mellifera carnica* compared to *A. mellifera capensis* were found and *A. mellifera ligustica* compared to *A. mellifera monticola* hybrids. A shorter development time in bees is a time factor limiting the reproduction rate of *V. jacobsoni* because it would prevent the mite from completing its development (Wilde and Koeniger 1992). Selection for a post-capping period, that is even a few hours shorter, may decrease the development of *V. jacobsoni* infestations. The grooming behaviour towards phoretic *V. jacobsoni* mites seems to exist to a lower degree in *A. mellifera* compared to *A. cerana*. It is evident that *A. mellifera* is able to kill *V. jacobsoni* mites (Ruttner and Hanel 1992). The effect of this defense behaviour on the development of the colony infestation is still unknown. *A. mellifera* from Europe and North America stocks also remove *V. jacobsoni* from capped brood cells. However, in some cases, the caps of mite-infested brood are opened and then closed again without eliminating the parasitized brood. Mite removal may limit the Varroa population because immature mites are killed, female mites are killed and those that survive the removal process cannot reproduce. *A. mellifera* removes not only mite-infested drone brood but also worker brood, which is in contrast to the behaviour of *A. cerana* (Boecking 1994).

20.4.1 Resistance Mechanisms and Factors of the Honeybee as a Social Insect

Analysis of the immune responses in the bees revealed the well developed system through which the individual bee acquires a special type of immunity. This system is based on cell-mediated reactions and a number of innate and inducible immune proteins, some of them potent antibacterial proteins like lysozyme and apidaecin-family peptides. Apart from internal immune defenses, other mechanisms of the colony resistance to invaders are known. They include the antibacterial activity of honey, nectar and pollen, secretions of the honeybee exocrine glands, antimicrobial activity of propolis. A specific behaviour resistance protects the bee colony from bacterial and fungal infections. The antimicrobial activity of honey, nectar and pollen is an important factor in the colony that inhibits the development of many saprophytic bacteria and fungi in stored food, and that could destroy some pathogenic micro-organisms (Burgett 1978). The acidity, osmotic pressure, and production and accumulation of hydrogen peroxide are responsible for this effect in honey and nectar (White and Subers 1963). Honey as a hyperosmotic medium may kill many living cells, except those of osmophilic fungi and bacteria.

Secretions from honeybee exocrine glands contain biologically significant components. The hypopharyngeal gland secretions of young workers contain proteins to be bacteriostatic and bactericidal to a wide range of bacterial species (Rose and Briggs 1969). At least two bacterial inhibitors are identified in royal jelly: 10-hydroxy-2-decenoic acid and glucose oxidase. It can also inhibit or delay the growth of many

fungi, for example *A. Apis*. Propolis, that is a highly complex mixture of waxes, resins, balsams, oils and a small amount of pollen, forms a part of antimicrobial defense of the bee colony. Flavanones together with flavones, caffeic acid and its esters are considered to be responsible for antibacterial action of propolis (Greeneway et al. 1990). It is quite possible that fungi of plant origin and from animal sources, polluting environment and contaminating pollen sources and water gathered by bees are inhibited by biologically active compounds of propolis.

20.4.2 Mechanisms to Reduce Diseases and Parasites

Feral and domesticated honeybee colonies have evolved elaborate defense mechanisms to protect both themselves and their food from pathogen and parasite invasion. The defense mechanisms of individual bees serve to minimize the threat for the entire colony. Constitutional defense mechanisms, such as the chitinous cuticle, which serves as a barrier between internal and external environments, and the intestinal microflora of the bee gut, can protect each individual bee against infectious diseases (Dustmann 1993; Gilliam 1993; Gilliam et al. 1988, Gliński and Jarosz 1995; Jacobs et al. 1990, Mitro 1993a, b). Cellular defense mechanisms (hemocytes) and humoral reactions (enzyme and antimicrobial factors) can contribute to resistance towards infections (Casteels et al. 1985; Jacobs et al. 1990; Mitro 1993a, b, 1994; Van Steenkiste 1988; Wienands and Madel 1987). The proventricular valve enables the bees to filter ingested spores, which serves as a mechanism of physiological resistance to diseases (Dustmann 1993; Sturtevant and Revell 1953). These individual responses, coupled with the bees' short life-span and rapid replacement with healthy individuals, can limit the spread of infections between bees within a colony.

Of interest to this article are the behavioural defenses that limit the spread of diseases and parasites. Hygienic and grooming behaviours are examples of such behavioural defenses. Hygienic behaviour is the main mechanism by which *A. mellifera* resists the brood diseases American foulbrood (AFB) (*Paenibacillus larvae* larvae) (Rothenbuhler 1964; Sturtevant and Revell 1953) and chalkbrood (*Ascosphaera Apis*) (Gilliam et al. 1983, 1988; Spivak and Gilliam 1993). Hygienic honeybee workers have the ability to detect diseased brood, uncap the wax covering over the brood cells and remove the infected larvae or pupae. The impact of hygienic behaviour on the spread of infective diseases is maximized if it takes place before the causative organism reaches the infectious sporulating stage (Spivak and Gilliam 1998a, b; Woodrow and Holst 1942).

Hygienic behaviour of worker honeybees is determined largely by two behavioural components, the uncapping and the removal of dead brood. Few colonies (10 % or less) in nature demonstrate hygienic behaviour (Olroyd 1996; Spivak and Gilliam 1991, 1993). However, colonies can be readily selected for the behaviour using a field assay (Spivak and Gilliam 1998a, b), or by direct inoculation with the pathogen (Gilliam et al. 1988; Rothenbuhler 1964) or mites (Boecking and Drescher 1990; Spivak 1996).

Grooming behaviour enables individuals and groups of bees within a colony to remove dust and pollen from their bodies, to disperse pheromones, and to remove ectoparasites. Grooming behaviour involves biting and licking with the mouthparts and movements of the mesothoracic legs. Antennae are carefully cleaned by the antenna cleaner on the prothoracic legs (Bozic and Valentincic 1995; Jander 1976; Winston 1987).

Many self-grooming (auto-grooming) activities of bees can be seen on flowers, in flight, during pollen collection and in the hive. Social grooming (allo-grooming) between bees can be observed within the hive. Allogrooming may be elicited by a grooming dance, first described in *A. mellifera* by Haydak (Haydak 1945). A bee performing this dance is groomed by a nestmate with the mesothoracic legs (Milum 1955). Grooming is an important mechanism of protection against the invasion of tracheal mites (*Acarapis woodi*) into the tracheal tubes in bees known to have genetically based resistance to this parasite (Danka and Villa 1998; Lee 1963; Pettis and Pankiw 1998).

20.4.3 Defense Mechanisms of *A. cerana* to *V. Jacobsoni*

The original host of *V. jacobsoni*, *A. cerana*, lives in equilibrium with this ectoparasitic mite. The balanced host-parasite relationship between *A. cerana* and *V. jacobsoni* can be explained by the limited reproduction of the mite on this bee species and behavioural defense mechanisms of the bees against the mites. The removal of mite-infested worker brood, or hygienic behaviour, is one defense mechanism of *A. cerana* towards *V. jacobsoni* (Peng et al. 1987b; Rath and Drescher 1990a). In some cases, *A. cerana* workers open the cappings of mite-infested brood without removing the bee brood. The mites then leave the opened cells or are removed by the bees, and the brood cells are subsequently resealed with a new wax cap (Rath 1991; Rath and Drescher 1990a; Rosenkranz and Tewarson 1992; Rosenkranz et al. 1993; Tewarson et al. 1992). Investigations by Rosenkranz et al. (1993) indicated that the number of infested pupae that are removed from experimentally infested cells may be lower than previously reported (Peng et al. 1987b) because the source of mites used for experimental infestation influences the removal behaviour of the bees. When mites from other colonies (either intraspecific *A. cerana*, or interspecific *A. mellifera*) were introduced into the brood cells, the removal rates of mite-infested brood were higher (about 60 %) than when the introduced mites were collected from within the test colony itself (about 10 %). *A. cerana* workers do not remove mite-infested drone brood owing to the thick cell capping over the drone cell, a structure unique to *A. koschevnikovi* and this species. Drones which are infested with multiple mites become weakened and are not able to open their cell caps from the inside as they normally do at the time of emergence. They die together with the mites inside such cells because the worker bees do not open these cells from outside, thus creating 'mite-traps' in the brood nest (Rath 1991). Initial infestation rates with two mother mites per cell can reduce the emergence rate of the infested drones to less than 30 %. Recently Boecking found that *A. cerana* bees sometimes additionally close the central pore in the drone cell cap of such infested pupae from outside with wax material,

entombing the mites within the brood nest. Consequently the successful reproduction of *V. jacobsoni* in *A. cerana* drone brood is limited, resulting in low overall rates of parasitism.

Grooming behaviour towards phoretic mites is another known defense mechanism of the Asian bee towards the ectoparasitic mite (Büchler et al. 1992). Auto- and allo-grooming behaviour of *A. cerana* workers is dependent on the ability of the workers to detect the mite and successfully groom it from the bee's body. The bees can readily be observed grabbing and crushing mites in their mandibles. If a bee fails to groom herself, she may perform the grooming dance by rapidly shaking her abdomen. The dance elicits allo-grooming by nestmates (Peng et al. 1987b). Thus, mites are disturbed and sometimes killed by the grooming behaviour of *A. cerana* workers.

Investigations by Fries et al. (1996) indicate that this behaviour may be less effective in combating mites than previously reported (Büchler et al. 1992). Using *A. cerana* and *A. mellifera* bees in cage experiments, observation hives and full-size colonies, the authors found that the proportion of live mites that had visible damage as a consequence of grooming was about 30 % in *A. cerana* compared to 12.5 % in *A. mellifera* colonies. Fries et al. (1996) considered only instances of successful grooming in which the mites were damaged, in contrast to Peng et al. (1987b) and Büchler (Büchler et al. 1992), who included the movement of mites from one bee to another bee, and the observer losing sight of the mites as grooming events.

V. jacobsoni mites are specifically adapted to their host, having distinct attachment sites on the bees' bodies which make it difficult for the bees to successfully groom the mites (Rath 1993). If not groomed off, the mites can survive on the bees for long periods without reproduction (several months see Rath (1991) and can disperse to other colonies while attached to drifting bees. Infestation levels of up to several hundred adult female mites in *A. cerana* colonies demonstrate that *V. jacobsoni* does survive in the colonies and is well adapted to its original host (Anderson 1994; Rath 1991, 1993). It should be emphasized that, in principle, grooming and hygienic behaviours of *A. cerana* towards *V. jacobsoni* are factors which contribute to a balanced host-parasite relationship. However, because the mite does not reproduce, or has very limited reproduction in worker brood of *A. cerana*, grooming and removal behaviours may not be the most important mechanisms of resistance. Based on simulation studies on the population growth of the mite, Fries et al. (1994) argued that the limited reproduction of *V. jacobsoni* on seasonally occurring drone brood in *A. cerana* is sufficient to explain the bees' tolerance of the mite.

20.4.4 Hygienic Behaviour and Grooming Behaviour as Defense Mechanism

20.4.4.1 Hygienic Behaviour

Boecking and Spivak (1999) studied the hygienic behaviour and grooming behaviour as mechanisms of defense against brood diseases and parasitic mites. Hygienic behaviour can be characterized by the rapid detection of sick and dead brood by worker bees, removal of dead insects from the colony and the thorough cleaning of the cell of

honey comb. Worker bees groom their own bodies and those of other bees, maintain the hygiene of the nest and remove debris from the hive. This hygienic activity is important in the resistance to chalkbrood and stone brood. The adults remove the mummified larvae using their mandibles and carry the larvae away from the nest. Bees that have no means of removing the pathogenic fungi from the gut and the body hair, subsequently reinfect susceptible larvae when feeding them or pass on infectious fungal spores to other adults of the colony (Southwick 1994). Resistance is supported by an ability of some worker bees to filter ingested spores and mycelial fragments from the proventriculus. Inhibitors in the glandular-produced brood food are strong antibacterial and antifungal agents.

There are at least two mechanisms of behavioural resistance; both are genetic in nature. Hygienic behaviour is believed to be controlled by two recessive genes, one for uncapping diseased brood and other for the removal of mummy (Taber 1992). The expression of hygienic behaviour depends on the strength of the bee colony. When colony size is reduced by removing frames of brood and associated bees, hygienic activity is depressed in hygienic colonies but there is no effect in non-hygienic colonies. The expression of hygienic behaviour is also altered by adding hygienic or non-hygienic bees to colony, and by the colony composition. Taber (1992) has stated that all bees with hygienic behaviour tested to chalkbrood were resistant. Southwick (1994), however, has suggested that there is not straightforward correlation between hygienic behaviour and resistance to chalkbrood. The chalkbrood infected colonies showed a weak correlation with hygienic behaviour.

Studies revealed that *A. mellifera* colonies remove worker brood infested with *Varroa jacobsoni* mites from the nest (hygienic behaviour), and groom the mites off other adult bees, but to a limited extent compared to the original host of *V. jacobsoni*, *A. cerana*. Bees infested with multiple mites during their metamorphosis show degenerated fat bodies, underdeveloped hypopharyngeal glands and a shortened lifespan (Beetsma et al. 1989; De Jong et al. 1982; Schatton-Gademayer and Engels 1988; Schneider and Drescher 1987). In addition, secondary infections (viruses and bacteria) can be transferred by the mite or triggered in the bees' body and are the primary cause of bee mortality in colonies severely infested with *V. jacobsoni* (Allen and Ball 1996; Ball 1988, 1996; Hung et al. 1995, 1996; Koch and Ritter 1989; Pohl and Ritter 1997; Ponten and Ritter 1992; Shimanuki et al. 1994). Because *A. mellifera* colonies die from varroosis within a few years if the mite population growth is not regulated by the beekeeper (Bitterman et al. 1983; Allen and Ball 1996; Rosenkranz et al. 1985) and chemical control has its problems and limitations (Milani 1999; Wallner 1999), it is of common interest to breed bees for a higher resistance to this mite. Selection and breeding are long-term solutions to the present crisis in apiculture. Szabo and Walker (1995) stated that this solution "... is the most complex, time consuming, and economically significant research for the beekeeping industry". Using the known defense mechanisms of *A. cerana* Fabr. as a model, Boecking and Spivak (1999) studied the behavioural defense mechanisms of the honeybee *A. mellifera* to the mite *V. jacobsoni*.

Hygienic behaviour is a mechanism of resistance to American foulbrood (Spivak and Reuter 1997), chalk brood (Gilliam et al. 1983) and *V. destructor* (Boecking

and Drescher 1992). The hygienic behaviour of honeybees is a defensive mechanism including uncapping brood cells and removing diseased or dead brood. Hygienic behaviour is genetically determined (Moritz 1988), however, Spivak and Gilliam (1993) suggested it is also affected by colony strength and composition of workers within the colony. Hygienic behaviour is tested by several methods including removing artificially infested brood with *V. destructor*, removing freeze killed brood or pin killed brood after some period. Workers removed double infested brood and freeze killed brood more frequently than single infested brood (Boecking and Drescher 1992). *A. cerana* is very effective in detecting and removing mites in worker brood (Peng et al. 1987b; Rath and Drescher 1990a). *Cerana* workers are not inclined to remove infested drone brood but in four days remove almost all mites (94 %) in worker brood (Rath and Drescher 1990a). In contrast, *A. mellifera* in general showed lower removal response toward worker infested brood than *A. cerana*. Boecking and Drescher (1992) reported a removal rate of 24 % in single infested cells and 41 % removal response in double infested cells. However, there is high variability in removal responses between strains of *A. mellifera*, ranging from 5.5 to 96 % in single infested cells and 5 to 100 % in double infested worker cells (Boecking and Drescher 1992). Janmaat and Winston (2000) reported the influence of pollen storage on removal rate of workers infested with *V. destructor*. Low pollen colonies with a high demand for pollen performed high foraging that leads to a reduction in the number of bees engaged in cell removal behaviour. Spivak and Reuter (1997) demonstrated that colonies established with open mated queens from hygienic stock had greater hygienic behaviour than unselected stock. In the process of uncapping and removing brood, immature female mites die (Spivak 1996) and adult female mites could be damaged or killed (Boecking and Drescher 1992). The main effect of hygienic behaviour is to postpone the phoretic period. Escaped mites may re-enter another available brood or adhere to adult bees (Boecking 1994). Mangum et al. (1997), in modelling population biology and population genetic dynamics of the honeybee with respect to the mite, estimated that the frequency of hygienic behaviour is insufficient to protect a colony from *Varroa* mite population growth.

20.4.4.2 Grooming Behaviour

Studies on the genus *Varroa*'s original host, *A. cerana*, revealed that adult bees perform a grooming behaviour when mites are deposited on their thoraxes (Peng et al. 1987b; Bozic and Valentincic 1995). Bees may engage in autogrooming behaviour in which they brush their entire body with their legs, while twisting their abdomen. If the mite is not removed immediately, it may hide in the propodeum. The bee may begin dancing to induce allo-grooming behaviour in which one or more bees look for the mite on the infested bee, pick up the mite with their mandibles, and eventually drop it on the hive floor. Correlatively, a high proportion of the dead mites collected on the hive floor are mutilated. However, it appears that most mites only change host bee, but are not killed or removed from the hive (Fries et al. 1996). Although the impact of this behaviour on mite population dynamics remains unclear, it may partly explain AHB resistance.

Grooming behaviour allows bees to remove mites from their bodies and was well described by Peng et al. (1987b). Grooming behaviour includes self- grooming and nest-mate grooming performed by uninfested workers. The infested worker which could not remove the mite by itself performs the grooming dance by vibrating its body laterally. Workers triggered by the dance approach the bee, which stops dancing and stretches the wings and legs and raises up its thorax and abdomen. The nest-mates examine the body with antennae and remove the mite with the mandibles. In the process of removing mites from the bee's body, mites could be damaged (Peng et al. 1987b). Many authors have compared grooming behaviour between the Asian bee, *A. cerana* and the Western bee, *A. mellifera*. Regardless of the differences in the proportion of mites removed by bees, all reports are conclusive that *A. cerana* showed more intensive grooming behaviour than *A. mellifera* (Peng et al. 1987b; Büchler et al. 1992; Fries et al. 1996). *A. cerana* is capable of removing almost all mites, compared to *A. mellifera* which removes only a small portion of mites (Peng et al. 1987b; Büchler et al. 1992). In addition, significantly more mites were injured by *A. cerana* than *A. mellifera* and consequently fewer mites recover (Peng et al. 1987b; Büchler et al. 1992; Fries et al. 1996). There is no direct evidence that grooming under natural conditions in *A. cerana* plays a significant role in resistance to *Varroa destructor* (Boecking et al. 1993).

Pronounced grooming behaviour after half an hour of inoculation with the *Varroa* mite was reported in Africanized bees, which removed 38 % in the contrast to *A. mellifera* which removed only 5.7 % (Moretto et al. 1991b). A negative correlation between mite population growth and the number of injured mites found by Arechavaleta-Valasco and Guzman-Novoa (2001) and Moosbeckhofer (1992) suggests that grooming may play an important role in reducing population growth of *V. destructor*.

20.4.4.3 Removal Behaviour

Removal, or hygienic behaviour, involves the detection and removal of diseased brood. This behaviour has been shown to be a behavioural mechanism of resistance to the bacterial disease, American foulbrood (Rothenbuhler 1964) and some honeybees also remove brood infested with *V. destructor*. In naturally infested *A. cerana* colonies, bees remove a significant proportion of the mite infested brood (Peng et al. 1987b). When brood is artificially infested, bees remove only infested worker brood but not infested drone brood (Rath and Drescher 1990a). In one experimental infestation, *A. cerana* bees removed more than 50 % of the larvae in cells infested with mites collected from *A. mellifera* colonies (Rosenkranz et al. 1993). However, if introduced mites had been washed with ethanol and pentane, bees detected and emptied only 5 % of infested cells (though a further 22.5 % of cells were opened, and after removal of the mites the cells were recapped). This suggests that mite odour permits the bees to detect and remove infested brood. Although *A. mellifera* workers remove less than 10 % of cells artificially infested with mites collected in *A. cerana* colonies, they may remove a higher percentage of mites collected from *A. mellifera*

colonies (reviewed in Spivak 1996; Spivak and Gilliam 1998a, b; Boecking and Spivak 1999). Such removal behaviour could explain the low compatibility between AHB and the mite. Vandame et al. (2000) showed that the ability to resist *V. destructor* by honeybees was dependent on mite density, implying the existence of a threshold in the expression of removal behaviour. They also observed a higher mortality of the population of brood mites in Africanized than in European colonies, suggesting the importance of removal behaviour as an incompatibility factor. In this experiment, we tested whether grooming or removal behaviour significantly reduce the compatibility of *V. destructor* in AHB colonies. Based on the results, we attempted to quantify the costs and benefits of the behaviours to the bees.

Our results suggest that the three species of honeybees in the study had varied degrees of behavioural resistance to Tropilaelaps in the phoretic stage. *A. cerana* appeared to have the highest behavioural resistance owing to its body shaking in response to the presence of mites. Body shaking may be an effective response to Varroa which serves as a pre-adaptation and is still more effective against Tropilaelaps owing to its morphology. Tropilaelaps has a different morphology from that of Varroa and has probably not co-evolved with the behaviour. *A. dorsata*, the natural host of Tropilaelaps, also showed a high level of behavioural resistance to Tropilaelaps. However, since the mite has co-evolved with *A. dorsata*, it is more successful on *A. dorsata* than it is on *A. cerana*. Although *A. mellifera* has not been exposed to parasitic brood mites until its introduction to Asia, it does display auto-grooming behaviour in response to them. This is probably a pre-adaptation as a result of its co-evolution with Acar *Apis* mites. The success of *A. mellifera* auto-grooming is less than that of the two Asian bees. However, because *A. mellifera* was able to remove about 50 % of the mites after 12 h and injured at least 1/5 of the recovered mites, a selection program may result in a stock of *A. mellifera* that is resistant to *T. mercedesae*. *A. mellifera* varies in its resistance to *Acarapis woodi* with European *A. mellifera* having more resistance than North American *A. mellifera* (de Guzman et al. 1998; de Guzman et al. 2002). Lineages of *A. mellifera* that show increased resistance to *A. woodi* may be more resistant to Tropilaelaps.

20.5 Considerations and Unresolved Questions

Promoting natural resistance to *V. jacobsoni* through selection and breeding is in its infancy today. Despite the extensive research on grooming and hygienic behaviours, it is still unclear as to what extent they are effective mechanisms of defense against the mites. It is commonly thought that bees that vigorously shake and bite the mites are the most efficient groomers. However, this disturbance by the bees may cause the mites to invade brood cells more readily. This possibility remains to be tested. Likewise, it is thought that colonies that rapidly uncap and remove diseased and parasitized brood are more resistant to diseases and mites. It has been shown that the spread of infective diseases is minimized if hygienic bees remove the diseased brood before the causative organism reaches its infectious sporulating stage (Woodrow and

Holst 1942). However, Spivak and Gilliam (Spivak and Gilliam 1993) considered the possibility that non-hygienic behaviour in honeybees could benefit a colony. If bees leave diseased brood under a capped cell, they may avoid contact with the pathogens in the cell. It is not clear if non-hygienic bees actively avoid diseased brood, or if they do not respond to it. Bees from pre-selected non-hygienic colonies tended to recap partially uncapped cells that contained freeze-killed brood (Spivak and Gilliam 1993). *A. mellifera* bees also recapped mite infested brood cells that had been previously uncapped by other bees (Boecking 1994). In *A. cerana*, this non-removal behaviour is much more marked (Boecking 1994); they uncap and remove worker brood cells infested with *V. jacobsoni*, but tend not to remove drone brood infested with multiple mites or with bacteria disease. The bees close the pore of the drone cell cap with wax from the outside. The non-removal of infested drone brood and closure of the central pore by the workers isolates the mites and infectious diseased cells in the brood nest.

When *A. mellifera* does remove mite infested brood, it is not clear at what point the bees should perform this behaviour to most effectively limit the population growth of *V. jacobsoni*. To negatively affect the potential average number of offspring per mother mite, removal should begin after the foundress has laid her full clutch of eggs in the brood cell. At this point, the bees would destroy the offspring during removal of the pupae, and the foundress would have to enter the phoretic stage again before she could invade a new brood cell. In highly infested colonies, it may not be advantageous for the bees to remove all miteinfested worker brood, which could substantially reduce the adult population of the colony.

Hygienic behaviour of honeybees may be a generalized adaptation for cell reuse and thus, may present a conflict between risking infection by removing diseased brood and the need to clear out comb space for the queen to lay eggs (Spivak and Gilliam 1993). *A. cerana* can use another strategy to avoid mites; they tend to abscond from the nest when under high disease or parasite pressure. Predators (such as wax moths) then destroy the abandoned combs, ridding them of the infectious material. Lines of *A. mellifera* demonstrate different degrees of susceptibility to *V. jacobsoni* (Büchler 1990, 1993, 1997; De Guzman et al. 1996; Harbo 1996; Harbo et al. 1997; Pechhacker and Zeitgemä 1995). When colonies are initiated with equal number of mites, the most resistant colonies can be identified as those that have the fewest mites at the end of the test period (Bienefeld 1996; Büchler 1993). However, numerical data on just the population growth or decline of the mites during the experimental period do not yield information on the mechanisms that influenced these trends. In breeding bees for mite resistance, it is critical to determine the exact nature of the traits that confer resistance, and to ensure they are measurable and heritable (e.g., Harbo and Harris 1999; Harbo et al. 1997).

The main genetic parameters which influences the bees' expression of hygienic behaviour are maternal and heterotic effects which dominate additive gene effects (Hoffmann 1996). In contrast, grooming behaviour is mainly influenced by additive gene effects, such that a combination of specific maternal and paternal bee lines is necessary for the breeding of mite-resistant stock. Such a combination that favors hygienic behaviour will not likewise favor grooming ability and vice versa

(Hoffmann 1996). It has been documented that colonies that show a high degree of removal of mite-infested brood are not necessarily better groomers (Spivak 1996).

High genetic variability and heterosis do not seem to enhance resistance to *V. jacobsoni*, although these factors do increase colony productivity (Fuchs et al. 1996; Neumann and Moritz 1996). In a 2-year hybrid queen breeding program, characteristics related to disease resistance and in particular to resistance to *V. jacobsoni* (infestation level, duration of the post-capping brood phase, hygienic behaviour and number of damaged mites), line-mixed colonies showed a consistently lower performance compared to the separate lines. This result is contrary to the theoretical expectation of enhanced resistance to diseases with increased genetic variation between workers (Fuchs et al. 1996).

In conclusion, bee breeding strategies for increased resistance to *V. jacobsoni* should be based on knowledge of the mechanisms of resistance, their potential for decreasing mite population growth and their mode of inheritance. At the same time, the ability of the mite to adapt to changes in their host should be taken into consideration.

20.6 Death of Mites Within Colonies

The rate of death obviously has a negative influence on the population growth. Despite protection of the mite inside the brood cell, a small portion of mites (1–3 %) fail to escape from bee food in which it was immersed and so become entombed between the cell wall and the cocoon. Further death of mother mites (1–5 %) could occur during reproduction (Martin 2001a). Mite offspring suffer much higher mortality. The greatest juvenile mite mortality occurs at the latest stage of mite development (immobile phase—deutochrysalis) ranging from 16 % in the first offspring to 60 % in the third offspring in *A. mellifera macedonica* (Infantidis et al. 1999). Considering the fourth offspring which could not complete development and therefore dies upon bee emergence, the total proportion of offspring that die within the cell would be even higher. Mechanical factors such as a movement of a moulting pupa or a bee within the cell could kill developing mites, but only the third or fourth daughter mite in a sequence. The increase in mortality rate of the second offspring compared to the first clearly indicates the presence of other factors influencing death of juvenile mites within the cells (Infantidis et al. 1999).

The emergence of bees corresponds to the number of dead mites registered in debris counts on the bottom of the colony (Lobb and Martin 1997). The mite fall in hatched worker brood is 2–3 times higher than in drone brood, probably due to shorter development time of workers barely allowing complete development of the last female offspring (Lobb and Martin 1997). Lobb and Martin (1997) estimated that half of the mites that fall from the nest to the bottom of the colony include those that die within the cells and those which die after emergence due to incomplete development. The other half of fallen mites was still alive and could reproduce when artificially introduced to brood. This portion of mites could represent those that fail to successfully change a bee host. Two days after emergence of infested bees most of the mites change their host (Kovac and Crailsheim 1988).

The life expectancy of *V. destructor* under natural conditions depends on the biology of a colony. In the period with brood it is estimated to be about 27 days and in broodless winter period it exceeds 5–6 months (Martin 2001a). Bowen-Walker and Gunn (1998) showed that mites fallen from the winter cluster with dead bees could survive up to 48 h by feeding on dead bees. Most mites (75 %) effectively left dying or dead bees and found a new host in the winter cluster within 24 h. This clearly indicates that mites move between hosts and on the other hand that the survival of mites in the winter cluster is not completely related to death of bees and therefore it is higher than expected (Bowen-Walker and Gunn 1998). In contrast, Fries and Perez-Escala (2001) could not find that mites become concentrated on the remaining bees in the winter cluster as the number of mites per dead bee was not significantly different from the number of mites per living bee. This difference in results is probably due to different techniques used which result in different opportunities for mites to re-infest adult bees after falling from the winter cluster. Mite mortality is monitored by counting fallen mites in debris. This method is recognized as a useful parameter to estimate population levels in honeybee colonies by many authors (Liebig et al. 1984; Calatayud and Verdu 1993; Fries et al. 1991a) except for colonies with well expressed grooming behaviour (Arechavaleta-Velasco and Guzman-Novoa 2001). Nevertheless, the parameter is highly variable and depends on season and the presence of brood and gives rougher estimates of a mite population within the colony. The estimates are most accurate during the broodless period and the period in which colonies have large brood nests (Martin 1998). The model of Martin (1998) accounts for seasonal differences in brood amount by using different multiplication factors to convert the daily mite mortality into an estimate of the mite population within the colony.

20.7 Factors Outside the Colony That Influence Population Dynamics of *V. Destructor*

Factors such as spread of mites and mite mortality outside the colony influence population dynamics of the mite. Despite the importance of these factors in population dynamics, both the spread of mites and mite mortality have not received much attention. Most research has covered spread of mites which contributes to invasion of large areas by mites, while the mite mortality outside the colony due to foraging has been poorly researched and therefore is still not completely understood.

20.7.1 Spread of Mites

The spread of the mites has a great impact on population dynamics of *V. destructor* and its virulence. The fitness and virulence of the mite does not depend only on the ability of mites to reproduce and spread within the colony but also on the ability to

spread between colonies. The mite *Varroa destructor*, an ectoparasite of *A. cerana* and *A. mellifera* brood and adults, was previously limited to only some regions of the world where it parasitized its single original host, i.e., the species *cerana*, without causing damage to apiculture. The parasite–host relationship between *A. cerana* and varroa seemed to have reached equilibrium because of the development of defense mechanisms by this bee species against the parasite (Peng et al. 1987b).

20.8 Behavioural Strategies Against Predators

Woyke et al. (2008) reported that unlike the more recent cave-dwelling honeybees viz., *A. cerana* and *A. mellifera*, giant honeybees which build their nests predominantly in the open are directly exposed to a variety of predators, particularly to birds and wasps. This predatory pressure apparently gave rise to the evolution of a series of defense strategies. Generally, defense behaviours of honeybees may involve physical contact with aggressors. A prominent example is their capacity to recruit stinging guards and to mobilize a whole army of defenders. In Giant honeybees, mass mobilization of stinging guards may occur within a split second, and gave them the reputation of being the most dangerous stinging insects on earth. Against wasps, which are major predators of bees, honeybees have developed specific defense behaviours such as heat balling of wasps that come into direct contact with the honeybee nest. Heat balling has been reported for *A. cerana*, *A. mellifera* and *A. dorsata*. For this purpose, the bees heat their thoraces by their flight muscles to above 45 °C, a temperature that is lethal to wasps.

Honeybee colonies also defend themselves without physical contact with their enemies, minimizing the risk for the defending bees by their behaviours such as the colony aggregation and shimmering behaviour. Kastberger et al. (2008) reported that Giant honeybees (*Apis dorsata*) which nest in the open have evolved a plethora of defense behaviours against predatory wasps, including hornets. They display highly coordinated Mexican wave-like cascades termed ‘shimmering’. Shimmering starts at distinct spots on the nest surface and then spreads across the nest within a split second whereby hundreds of individual bees flip their abdomens upwards. The findings suggest that shimmering creates a ‘shelter zone’ of around 50 cm that prevents predatory wasps from foraging bees directly from the nest surface. Thus shimmering appears to be a key defense strategy that supports the Giant honeybees’ open-nesting lifestyle.

Shimmering has been observed in *A. cerana* and *A. florea* (Oldroyd and Wongsiri 2006), and in *A. dorsata*, it is a notable visual cue that is impressive even to humans (Roepke 1930), Seeley et al. 1982). Japanese honeybees, *A. cerana japonica*, guard against their local, predatory hornets, *Vespa simillima xanthoptera*, in a different way. Both the bees and the hornets are accustomed to a relatively cool climate, and Masato Ono et al. found that when bees are threatened by a hornet, a huge group of bees thermoballs it, surrounding the predatory hornet in a cluster and vibrating their

muscles until they heat the hornet to a temperature that kills it (113 °F, 45 °C). This form of thermal strategy seems to be a rare type of colony.

In similar studies, Woyke et al. (2008) observed the defense behaviour of three, free living giant (Meg *Apis*) honeybee subspecies, *Apis laboriosa*, *A. dorsata dorsata* and *A. dorsata breviligula*. They found that disturbed worker bees responded with characteristic dorso-ventral defense body twisting (DBT). Workers of *A. laboriosa* twisted the thorax by 55°, and the two other *A. dorsata* subspecies by about 10° more. *A. laboriosa* workers raised the tip of the abdomen by 90° and workers of the two other bee subspecies by about 20° higher. Differences in those traits were highly significant between *A. laboriosa* and both *A. dorsata* subspecies, but were not significant between those two subspecies. The whole cycle of DBT was the most vigorous in *A. d. breviligula* (0.11 s), and it was twice as vigorous as in *A. d. dorsata* (0.26 s) and thrice as in *A. laboriosa* (0.32 s). *A. laboriosa* twisted the body together with wings folded over the abdomen, while the two *A. dorsata* subspecies raised the abdomen between spread wings. This supports the opinion to treat *A. laboriosa* as a separate species.

20.8.1 Bee Wasp Interactions

Predatory wasps pose a serious threat to apicultural industry in different parts of the world. A persistent attack of predatory wasps weakens the bee colonies and most often the colonies either perish or abscond (Adlakha 1975; Gupta and Das 1977; Singh 1972; Sharma and Raj 1988; Shah and Shah 1991). Many species of vespidae are serious enemies of honeybees and cause considerable damage (Mishra et al. 1989). A survey by Walton and Reid (1976) revealed that in 1975–76 *Vespula germanica* destroyed 3,900 colonies and affected more than 10,000 others. Akre and Davis (1978) reported that in Japan a group of 30 *Vespa mandarina* was able to kill 25,000 out of 30,000 bees in just three hours at the rate of one bee per hornet every 14 s. Sharma et al. in 1985 found that the wasp attack most often coincides with the floral dearth period and monsoon season. This is resulting in the depletion of colony strength and economically discouraging the beekeepers. The giant hornet (*Vespa magnifica*) and little hornet (*V. basalis*) were serious predators to honeybees in Nepal. In an apiary, two individual wasps killed an entire colony of *A. cerana* in an hour (Thapa et al. 2000). On an average, 20–25 % of bee colonies are lost due to persistent wasp attacks. The wasp's attacks usually coincide with the dearth periods when the bee forage sources such as nectar and pollen are scarce. Of all the *Vespa* species preying *A. mellifera* L. and *A. cerana* F., *V. cincta*, *V. velutina* and *V. basalis* are the most serious and caused heavy losses by feeding on adult bees, their brood and honey reserves. *A. mellifera* L. is relatively more susceptible to wasp attacks than *A. cerana* F. and predation often coincides with flowerless dry season (July–Oct). The *V. mandarina japonica* is the only hornet species which is known to attack en masse. First a single hornet kills a few bees and takes them to its nest to feed larvae; then it marks the site (e.g. hive) with a pheromone from its Venter Vachit glands. Wasps

hover near hive entrance and catch returning/outgoing foragers and they also catch bees as they forage on the flowers. Adult bees, bee brood, honey, pollen etc. represent a vast storehouse of energy for wasps. The deserted colonies perish due to starvation as the wasp attacks are often synchronized with the dearth period. Wasp attacks usually increase after the middle of August. However, the *Vespa mandarinia* attack and crush honeybees one after another with their mandibles until all the honeybees are wiped out, then carry away all of the larvae. The hornets would enter the nest, kill the bees, and take their bodies home to their young. After a few successful trips, hornets rub a pheromone on the nest that signals other hornets to attack. Therefore, in autumn, most beekeepers think it is quite normal that measures are needed to protect the honeybees from these natural enemies. They attach very often a wasp trap to the front of the hive entrance.

The species composition and activity of the wasp is different at different time periods of the year. Abrol and Kakroo (1998a) found that the wasp attacks peaked during July to September which most often coincided with the floral dearth period and monsoon season taking a heavy toll on honeybee colonies, particularly the foragers. The attack of wasp is also different at different time of the day. The activity of the wasp is maximum in morning and noon time than that of late day and evening. The abundance of different species of wasps varies in different areas of the country (Ranabhat and Tamrakar 2008).

A number of methods are available to control the wasps, which include the killing of wasps by flapping, poisoning by different baits, trapping, killing of queens, destruction of their nests by burning or poisoning of nests, etc., but these methods are either not cost effective or have environmental problems. As a rule of nature, each living organism has developed one or more strategies to defend against their enemies (Okada 1984; Matsuura 1973, 1988). In earlier studies, Ahmad et al. (1985) and Muzaffar and Ahmad (1986) reported that wasps inflicted heavier damage to *A. mellifera* colonies compared to *A. cerana* as the latter species defended strongly against their attacks. Ono et al. (1987) and Ichino and Okada (1994) reported that workers of *A. cerana japonica* showed a distinct balling reaction against workers of the predatory hornet *Vespa simillima xanthoptera* killing the hornets by heat and suffocation generated inside the ball. It was therefore felt desirable to evaluate the strategies, if any, adopted by the local honeybee *A. cerana* and the introduced bee *A. mellifera* against their enemies.

20.8.2 Defensive Behaviour of *A. cerana* F. Against Predatory Wasps

Ono et al. (1987) and Ichino and Okada (1994) reported that workers of *A. cerana japonica* showed a distinct balling reaction against workers of the predatory hornet *Vespa simillima xanthoptera* killing the hornets by heat and suffocation generated inside the ball. Abrol (2006) studied the defensive behaviour of *A. cerana* F. and *A. mellifera* against predatory wasps *Vespa velutina* and *Vespa magnifica*, and found

that *A. cerana* showed a well organized defense and killed more number of predatory wasps by exhibiting well organized balling behaviour as compared to *A. mellifera* L. The bee mortality was higher when fewer wasps visited the apiary due to an unorganized defense. However, when the intensity of attack was severe, fewer bees and more wasps were killed due to an organized defense.

The hornet *Vespa mandarina japonica* has evolved a group predation strategy against other social bees and wasps. A scout hornet first marks the location of the site with a pheromone and other hornets will later attack the prey en masse. However, *A. cerana japonica* appears to have co-evolved to detect this marking behaviour and may attack the lone scent-marking hornet en masse themselves. When this occurs, 400–500 bees will form a tight ball around the hornet, a behaviour the authors term “balling”. The large number of bees and the compactness of the ball means that the internal temperature of the ball, i.e., the hornet’s “ambient” temperature, is raised sufficiently high to kill the hornet but not the bees. The lethal temperature for the bees is 48–50 °C whereas for the hornet it is only 44–46 °C. In fact, the ball is used to form an oven to cook the hornet. Ono et al. (1995) also suggest that an alarm pheromone, isoamyl acetate, whose evaporation is aided by the high temperatures, may act as a recruitment signal for other bees to join the structure. This species of bee reacts in a similar manner to attacks from a solitary hunting hornet, *V. simillima xanthoptera* and although the balls only consist of around 180–300 bees, the effect is the same. Susan (2005) reported that at least two species of honeybees, the native *A. cerana* and the introduced European honeybee, *A. mellifera*, engulf a wasp in a living ball of defenders and heat the predator to death. Ono et al. (1987, 1995) however, on the other hand reported that Japanese honeybees (*A. cerana japonica*) do not sting a captured wasp, but it is rather the temperature within the ball that kills the hornet. The studies on indigenous honeybee *A. cerana* Abrol (2006), revealed that in addition to thermal defense it tries to sting repeatedly at the junction between thorax and abdomen immediately after capturing a hornet. As soon as the hornet was dead, the bees gradually dispersed from the ball. An occasional bee may then try to remove the corpse, but is usually unsuccessful. The native Asian bees were found to have better heat-balling tactics than the European imports do. The native bees gather one-and-a-half times as many individuals in their swarms as the European bees do. In earlier studies, Sakagami (1960) and Butler (1962) have also reported that *A. mellifera* bees are heavy and slow in movement and fall easy prey to enemies. A new study of heat balling has described a margin of safety for the defending bees. Ken et al. (2005) report that the native bees use heat-balling techniques that the European bees lack. That makes sense, the researchers say, since the Asian bees have long shared their range with the attacker wasp *Vespa velutina*, but the European bees became widespread in Asia only some 50 years ago and so have had much less time to adapt to this wasp. The defensive abilities of *A. cerana* and *A. mellifera* honeybee colonies (actively balling the wasps) was found to significantly reduce the foraging activity with increased wasp attack time. They further found that core temperatures in a ball around a live wasp of *A. cerana* were significantly higher than those of *A. mellifera*. The lethal thermal limits for *V. velutina*, *A. cerana* and *A. mellifera* were significantly different, so that both species of honeybees have a thermal safety factor

in heat-killing such wasp predators. The Japanese honeybee (*A. cerana japonica*) has evolved an ingenious method of defending against the much larger predator. When a hornet scout locates a Japanese honeybee hive and approaches the nest, the scout will emit specific pheromonal hunting signals. When the honeybees detect these pheromones, a hundred or so honeybees will gather near the entrance of the nest, apparently to draw the hornet further into the hive. As the hornet enters the nest, a large mob of about 500 honeybees surround the hornet, completely covering it and preventing it from moving, and begin quickly vibrating their flight muscles. This has the effect of raising the temperature of the honeybee mass to 47 °C. Though the honeybees can tolerate such a temperature, it is fatal to the intruder, which can handle a maximum temperature of about 45 °C, and is effectively baked to death by the large mass of vibrating bees. The wasps died at 45.7 °C, but the Asian honeybees survived temperatures up to 50.7 °C and the European bees survived up to 51.8 °C (Susan 2005). The studies show that *A. cerana* has a well-organized defense strategy against predatory wasps compared to *A. mellifera*.

20.8.3 Shimmering

Shimmering behaviour is observed in the eastern honeybee, *A. cerana*, the cavity-nesting Borneo bee, *A. nuluensis*, the dwarf bee, *A. florea*, and the rockbee, *A. dorsata* and is usually considered as a response to the danger posed to the colony by intruding predators or enemies. It consists of highly coordinated, simultaneous flipping of abdomens upwards by several bees on the comb surface, followed like the 'Mexican wave' by a similar flipping of abdomens of adjacent columns of bees, the wave of motion passing from one end of the comb to the other. Tan et al. (2005) recently made a detailed investigation of the hawking behaviour of *Vespa velutina* and the response of *A. cerana* and *A. mellifera* colonies in China. They found that both honeybee species recruited guard bees at the hive entrance to stave off the predating wasps, and *A. cerana* guard bees performed wing shimmering as a visual pattern disruption mechanism, which is not shown by *A. mellifera*. Defensive behaviour of the two honeybee species against predation by wasps is, according to these scientists, fundamentally different. This they suggest is a result of predator-prey co-evolution and mutual adaptation between *A. cerana* and *V. velutina*. Similar shimmering behaviour exhibited by *A. nuluensis* when preyed by wasps supports this view. In *A. dorsata* colonies shimmering behaviour is usually observed, when birds, wasps or hornets hover near the comb. Gerald Kastberger, Evelyn Schmelzer of the Institute of Zoology, University of Graz, Austria and Ilse Kranner of the Seed Conservation Department, Royal Botanic Gardens, Kew, UK investigated the prey and predator interaction, and particularly, the success of the rockbees in defending their nests against wasps and hornets by shimmering.

Rockbees have a single comb that is covered by several layers of bees forming a curtain over the comb. However, the abdominal flipping is performed only by bees of the outer or roofing layer of the curtain in all regions of the nest. According to

Kastberger and Biswas (1998) the abdominal movements of thousands of bees are tightly coordinated, and form a cascading colony response in temporal and spatial terms, emanating from one or more centres, radiating over the nest of several tens of thousands of bees within 1 s. Kastberger et al. (2008) had demonstrated the role of Nasanov scent as a superior pheromone for the control and promotion of individual defensive reaction, synchronizing and maximizing the defensive response on colony level.

Kastberger et al. (2008) showed that the shimmering waves repelled wasps near and at a close distance around the bee colony. Shimmering evolved mainly for colony defense in *A. dorsata*. This strategy of colony defense poses practically no risk to the defenders, consumes far less energy than emergency reactions that are released when defense turns to a matter of physical contact with the enemy, for example, heat-balling and stinging the wasps. Kastberger, Schmelzer and Kranner presented quantitative proof of the anti-predatory response of rockbees to the presence of hornets. Shimmering waves became stronger and more frequent the nearer a predator came to the colony and the faster the hornet flew there. The hornets too were more affected by shimmering the nearer they came to the bee colony. While local small-scale shimmering may confuse wasps, according to the researchers, big-scale shimmering that may spread over the entire nest does have the capacity to repel predatory wasps. This affect was observed only when the wasps were within a restricted limit away from the bee hive.

20.8.4 Defense Waving (DW)

Close observation of hornet hunting near the nest was not described. According to Seeley et al. (1982) “Whereas an *A. cerana* robber or a falling leaf triggers just one to three waves of shimmering, a *Vespa* wasp’s close approach, even one lasting just a few seconds, elicits 50 or more waves of shimmering”. This opinion is repeated in two Ruttner books (Ruttner 1988, 1992) and is generally accepted. However, close up observations (Kastberger et al. 2008) showed that the cause of the differences is not the type of the intruders, but the number of repeated disturbances. When a disturbing insect, bird or any other intruder just pass or disturbed the nest for short time, this triggers just one or few DW. However, a hornet hunting near the nest is hovering there for a long time. Each change of its position triggers a new DW. After the hornet flies away, no more DWs are performed. The reported continuation of DWs after the hornet left the nest are probably due to inability in seeing the hornet from long distance of 18 m to the observed nests.

Butler (1954) reported that individual worker honeybees in South Asia exhibit a remarkable defensive shimmering behaviour of shaking their bodies from side to side. Sakagami (1960); Schneider and Kloft (1971); and Koeniger and Fuchs (1975) described in detail the side-to-side body movements in *A. cerana*. Kastberger et al. (1998) found defensive vertical abdominal shaking of individual *A. dorsata* worker bees. Defensive behaviour of individual workers followed by neighboring bees results in circular defensive waves on the surface of the curtain of worker bees covering the nests (Roepke 1930).

20.8.5 *Shimmering in A. cerana*

Butler (1954) writes that honeybees in South Asia “exhibit a remarkable behaviour pattern when a hornet, ant, wax-moth or other intruder approaches their nests. They shake their bodies violently from side-to-side in concert, a behaviour pattern which is referred to as “shimmering behaviour”. Schneider and Kloft (1971) and Koeniger and Fuchs (1975) showed that the amplitude of the abdominal tip reaches in *A. cerana* 1 cm to the right and 1 cm to the left. Koeniger and Fuchs (1975) suggested that similar behaviour occurs in *A. dorsata*. Although, Kastberger et al. (2008) observed many times the side-to-side shakings called shimmering in *A. cerana*, they could never see movements in this direction performed by *A. laboriosa* or *A. dorsata*.

20.8.6 *Shimmering and Defense Waving*

Kastberger et al. (2008) pointed out that two different defense reactions exist in Asian honeybees. *A. cerana* moves the body side-to-side in lateral direction, which is called “shimmering” (Butler 1954). However, individuals of *A. laboriosa* and *A. dorsata* respond with defense body twisting in vertical direction. Thus the use of term “shimmering” is not appropriate neither for DBT of individual bees or for DW in *A. laboriosa* and *A. dorsata*. Defense body movements of *A. laboriosa* versus both subspecies of *A. dorsata*. *A. d. dorsata* and *A. d. breviligula* twisted the thorax and lifted the abdomen highly significantly more than *A. laboriosa*. However, no highly significant differences were detected between those two *A. dorsata* subspecies. *A. laboriosa* twisted the body together with wings folded over the abdomen, while both *A. dorsata* subspecies raised the abdomen between spread wings. Thus, *A. d. dorsata* and *A. d. breviligula* differ quantitatively in performing DBT. However, both subspecies differ from *A. laboriosa* qualitatively in performing DBT. Those differences support the opinion to treat *A. laboriosa* as a separate species.

20.9 Thermal Defense of Bees Against Predatory Wasps

When worker honeybees ball another bee, the temperature increases in the core of the bee ball (Esch 1960) as it also does in individual worker bees attacking predators (Heinrich 1979) and in guard bees of *A. mellifera* (Stabentheiner et al. 2002). Defensive bee balling is particularly pronounced in the Asian honeybees, *A. cerana* (Ono et al. 1987) in response to wasp attacks (Matsuura and Sakagami 1973; Ken Tan and Wang 2004). When a hornet persists in attack, several worker bees engulf it in a ball and kill it by raising the core temperature “heat-balling” the wasps (Matsuura and Sakagami 1973; Ono et al. 1987). While 20–30 % of a colony of *A. cerana* may succumb to the predations of the wasp *V. velutina*, losses are even greater in the introduced European honeybee, *A. mellifera* (Sakagami 1960; Liang Qun 2001). Ken et al. (2005) performed experiments on the defensiveness of two species of honeybees against *V. velutina* to (1) further develop a comparative ethology of these

interactions, (2) to assess inter- and intra-specific differences in honeybees and (3) to document the thermal characteristics of such encounters. The latter authors (Ken et al. 2005) studied the defensiveness of honeybee colonies of *A. cerana* and *A. mellifera* against predatory wasps, *Vespa velutina*, and false wasps. There were significantly more worker bees in balls of the former than latter. Core temperatures in a ball around a live wasp of *A. cerana* were significantly higher than those of *A. mellifera*, and also significantly more when exposed to false wasps. Core temperatures of bee balls exposed to false wasps were significantly lower than those exposed to *V. velutina* for both *A. cerana* and for *A. mellifera*. The lethal thermal limits for *V. velutina*, *A. cerana* and *A. mellifera* were significantly different, so that both species of honeybees have a thermal safety factor in heat-killing such wasp predators. During wasp attacks at the hives measured at 3, 6 and 12 min, the numbers of *A. cerana* and *A. cerana indica* bees continuing to forage were significantly reduced with increased wasp attack time. Tropical lowland *A. c. indica* reduced foraging rates significantly more than the highland *A. c. cerana* bees, but there was no significant effect on foraging by *A. mellifera*. The latency to recovery of honeybee foraging was significantly greater the longer the duration of wasp attacks. The results show remarkable thermal fine-tuning in a co-evolving predator-prey relationship.

20.9.1 Thermal Lethal Limits of *A. cerana*, *A. Mellifera* and *V. Velutina*

According to Ken et al. (2005) when the temperature reached 45 °C, three wasps were dead and the remaining seven at 46 °C, but all 20 honeybees were still alive. At 51 °C, two *A. mellifera* were dead and another eight at 52 °C; at 50 °C, three *A. cerana* were dead and another seven at 51 °C. The lethal thermal limit for *V. velutina* was 45.7 ± 0.48 °C, and for *A. cerana* and *A. mellifera* 50.7 ± 0.48 °C and 51.8 ± 0.42 °C, respectively.

20.9.2 Variation in Foraging Numbers

The numbers of foraging *A. c. cerana* and *A. c. indica* were significantly reduced with increasing exposure of the wasps. *A. cerana* from the tropical lowlands were more sensitive to wasp exposure than those from the temperate highlands (*A. c. indica*). Interestingly, *V. velutina* wasp exposure had no significant effect on the foraging levels of *A. mellifera* and false wasp exposure had no apparent effect on the foraging levels of *A. cerana*.

20.9.3 Variation in Latency to Foraging Recovery Times

The longer the duration of wasp exposure to *A. cerana*, the longer the recovery time to initial foraging levels. The number of *A. c. indica* foragers returned to initial level

sooner than in *A. c. cerana*. When exposure was only 3 min, foraging number was reduced but returned to the initial level after 3 min for *A. c. indica* and 4 min for *A. c. cerana*. When wasp exposure was increased to 6 min, foraging number declined more obviously, and returned to initial levels after 5–6 min. Exposed to wasps for 12 min, foraging ceased and only some bees balled the wasp. After the wasp was removed, bees began to fly out in small numbers, and were restored to undisturbed foraging levels after about 7 min for *A. c. indica* and 11 min for *A. c. cerana*.

The aggressive defensive behaviour of *A. cerana* is more efficient than that of *A. mellifera* colonies both in terms of recruited workers and balling temperature increases and passively in discontinuing foraging when under wasp attack. Due to a lack of co-evolutionary history, one might expect less efficient balling behaviour in a honeybee species such as *A. mellifera*, which is not sympatric with such vespine predators. However, the occurrence of thermal balling in defense could well predate speciation in *Apis* because it also occurs in *A. dorsata* (Kastberger and Stachl 2003), a clade remote from *A. cerana* and *A. mellifera* (Alexander 1991).

The final core temperature of the *A. cerana* balls rose faster and higher (1.3 and 1.8 °C) for both live and false wasps than in *A. mellifera*. During balling, high temperatures are generated by rapid increases in the metabolism of honeybee workers through non-shivering thermogenesis as in *A. dorsata* (Kastberger and Stachl 2003). The differences in colony defense among honeybee species indicate that those honeybee species (*cerana*, *dorsata*, *nuluensis*) sympatric with wasps have evolved an especially efficient colony level defense system.

The vespine wasp, *Vespa velutina*, specializes in hawking honeybee foragers returning to their nests, which was studied in China using native *A. cerana* and introduced *A. mellifera* colonies. When the wasps are hawking, *A. cerana* recruits threefold more guard bees to stave off predation than *A. mellifera*. The former also utilizes wing-shimmering as a visual pattern disruption mechanism, which is not shown by *A. mellifera*. *A. cerana* foragers halve the time of normal flight needed to dart into the nest entrance, while *A. mellifera* actually slows down in flight manoeuvres. *V. velutina* preferentially hawks *A. mellifera* foragers, when both *A. mellifera* and *A. cerana* occur in the same apiary. The pace of wasp-hawking was highest in mid-summer but the frequency of hawking wasps was three times higher at *A. mellifera* colonies than at the *A. cerana* colonies. The wasps were taking *A. mellifera* foragers at a frequency eightfold greater than *A. cerana* foragers. The final hawking success rates of the wasps were about three times higher for *A. mellifera* foragers than for *A. cerana*.

Vespa velutina Lepeletier (Hymenoptera: Vespidae), a vespine wasp endemic to southeast Asia, preys on honeybees, both the native *A. cerana cerana* F. (Hymenoptera: Apidae) as well as the introduced European *A. mellifera* (Hymenoptera: Apidae) (Matsuura and Yamane 1990; Tan et al. 2005, 2007, 2010). The wasps hawk (capture) foraging honeybees on the wing near honeybee colonies, and predation is especially fierce in autumn when *V. velutina* are most populous (Li 1993). While native *A. cerana* colonies have evolved defense strategies against *V. velutina* predation, the introduced *A. mellifera* sustains significantly greater losses than the former (Qun 2001; Tan et al. 2005, 2007). If *V. velutina* come close to a honeybee nest,

the guard bee cohort increases, shimmers their wings, and, if *V. velutina* persist, the guard bees launch strikes to kill them by heat-balling (Tan et al. 2005, 2007). Such endothermic heat is generated by the thoracic musculature (Esch 1960; Esch and Goller 1991; Stabentheiner et al. 2003; Kunieda et al. 2006) and facilitates pre-flight warm up (Krogh and Zeuthen 1941; Heinrich 1979; Esch et al. 1991), brood incubation (Bujok et al. 2002), heat balling (Ono et al. 1987; Tan et al. 2005, 2007), and other defensive contexts (Southwick and Moritz 1985). When many worker bees ball a wasp, they can kill it by raising the ball core temperature to 46° C in about 3 min (Matsuura and Sakagami 1973; Ono et al. 1987; Tan et al. 2005). However, the “resting” temperatures of guard bees at a hive entrance are considerably lower than those reached during heat balling. Because a “resting” guard bee has a temperature of about 24° C, which nearly doubles to 46° C during heat balling, the hypothesis for this study was that such a physiological thermal jump must be graded and ought to be reflected in more gradual changes in the transition from simply guarding to active heat balling. The sequence of changes in the behaviour and thoracic temperatures of guard bees was examined during the transition from “resting” to poised alert, to wing-shimmering, and finally, to heat-balling attacks by *A. cerana* and *A. mellifera* against *V. velutina* hawking at the hive entrance.

Although heat-balling wasps as such is well documented (Ono et al. 1987; Tan et al. 2005), the behavioural sequence of attracting additional recruits to the guard bee cohort, increased numbers of wing-shimmering guard bees (an average of 32.2 ± 3.2 bees/ball (Tan et al. 2005) that raise thoracic temperature prior to striking *V. velutina* have not been previously measured for either *A. cerana* or *A. mellifera*. Unalerted guard bees of both *A. cerana* and *A. mellifera* have relatively low thoracic temperatures, about 24° C, but when hawking *V. velutina* approach them, unlike *A. mellifera*, *A. cerana* guard bees are immediately alerted and begin body shaking and wing shimmering. Likewise, thoracic temperature rapidly increases some $5.4 \pm 1.9^\circ \text{C}$, and those guard bees with the higher thoracic temperatures more readily attack *V. velutina* than those at lower temperature. The wing shimmering behaviour is directly associated with increasing the guard bee cohort, and may be mediated by the simultaneous release of a pheromone. Because shimmering guard bees increase their surface temperatures during wing-shimmering, this would facilitate the dispersal of any recruiting pheromones (Stabentheiner et al. 2002).

Likewise, during fanning *A. cerana* face away from the nest entrance (Sakagami 1960), and this would direct any pheromonal plume backwards into the nest. However, it has been reported that *A. cerana* does not expose its Nasanov gland during shimmering (Koeniger et al. 1996). Wing-shimmering is also interpreted as an anti-predator visual pattern disruption mechanism, similar to that of *A. nuluensis* (Koeniger et al. 1996).

In contrast, *A. mellifera* guard bees do not exhibit these behavioural responses to hawking *V. velutina*, and there is no rapid elevation of thoracic temperature. This apparent inability to rapidly detect *V. velutina* and to respond defensively accounts for the greater *V. velutina* presence and hawking success rate at colonies of *A. mellifera* than *A. cerana* in autumn (Tan et al. 2007). Moreover, *A. cerana* may also withdraw into its nest or use wing-shimmering, traits absent from the behavioural repertoire of

A. mellifera. However, wasp-balling behaviour is exhibited by *A. mellifera cypria*, which apparently kills wasps by asphyxiation (Papachristoforou et al. 2007).

Kastberger and Stachl (2003) suggests that such anti-predator behavioural adaptations may be widespread between predators and honeybees in southeast Asia. Tan et al. (2012) reported that the vibration is visually striking, and is accompanied by a loud buzzing noise. *A. cerana* guards are more than capable of backing up their ISY (I see you) display with defensive action. If a hornet lands at the hive entrance despite the ISY display, it is pounced on by the *A. cerana* guards, which then form a dense ball of up to 500 bees around the hornet (Ono et al. 1995; Tan et al. 2010). The ball of bees kills the hornet with a combination of heat and suffocation (Ono et al. 1987, 1995; Ken et al. 2005). In contrast, the Western hive bee *A. mellifera*, which has not evolved in the presence of Asian hornets, does not produce the shaking display, and is much less effective at heat balling hornets (Ken et al. 2005). Instead, *A. mellifera* guards tend to approach hornets as individuals, and are thus more vulnerable to hornet predation than *A. cerana* guards.

Sugahara and Sakamoto (2009) found that giant hornets (*Vespa mandarinia japonica*) are killed in less than 10 min when they are trapped in a bee ball created by the Japanese honeybees *A. cerana japonica*, but their death cannot be solely accounted for by the elevated temperature in the bee ball. In controlled experiments, hornets can survive for 10 min at the temperature up to 47 °C, whereas the temperature inside the bee balls does not rise higher than 45.9 °C. They found that the CO₂ concentration inside the bee ball also reaches a maximum ($3.6 \pm 0.2\%$) in the initial 0–5 min phase after bee ball formation. The lethal temperature of the hornet (45–46 °C) under conditions of CO₂ concentration ($3.7 \pm 0.44\%$) produced using human expiratory air is almost the same as that in the bee ball. The lethal temperature of the honeybee is 50–51 °C under the same air conditions. They concluded that CO₂ produced inside the bee ball by honeybees is a major factor together with the temperature involved in defense against giant hornets.

Avoidance of predation by wasps in the two honeybee species is fundamentally different, suggesting substantial predator–prey co-evolution between *A. cerana* and *V. velutina*. While both species kill predatory wasps by heat-balling, *A. cerana* elevates its ball core temperature higher than does the *A. mellifera* and also contains more balling bees (Ono et al. 1987, 1995; Ken et al. 2005). In the presence of hawking wasps, *A. cerana* may also withdraw into its nest, which *A. mellifera* does not do. *A. cerana* guard bees also use wing shimmering as a visual pattern disruption mechanism, similar to *A. nuluensis* (Koeniger et al. 1996), which is absent from the behavioural repertoire of *A. mellifera*. These collectively executed timed waves of shimmering their wings, only when the nests are directly approached, appear to be a visual pattern disruption mechanism against predators. In any event, *V. velutina* preferentially hawk *A. mellifera* foragers when both *A. mellifera* and *A. cerana* occur in the same apiary.

Given wasp-hawking, returning *A. cerana* foragers make a very fast bee-line for the hive entrance to avoid the jinking wasps. *A. mellifera* slows down and sashays in the face of wasps. Clearly, this increases the exposure time to the predator and consequently results in more predation success. The success rate of wasps taking

A. mellifera foragers as prey at three times the rate of *A. cerana* losses is not so surprising, but it points to the fact that *A. cerana* has acquired abilities to partially thwart the predations of the wasps. In conclusion, our comparative study on the honeybees avoiding wasps' predation behaviour demonstrates that *A. cerana* can efficaciously escape from wasp's predation through changing flying behaviour, but *Apis mellifera* cannot.

20.10 Defensive Behaviour of Bees Against Wax Moths

Wax moths *Galleria melonella* and *Achroia grisella* are also victims of biting and dragging by bees within colonies (Eischen et al. 1986). These behavioural responses, which occur inside the nest, involve the same principles of enemy identification that apply to intruders at colony entrances and may be behaviourally related to threats from outside the nest. Eischen et al. (1986) found that Africanized honeybee colonies were quicker, more persistent and more intense in their attack than our European colonies against adults of greater wax moth and their hive entrances. The intensity of attack was related to the number of bees guarding the entrance and not with the colony size. Africanized honeybee has more number of guard bees as compared to European honeybees.

20.10.1 Prisons in the Bee Hive

Defensive strategies of bees against large parasites and pests:

Honeybee colonies also must defend themselves against a number of larger parasites and pests. Two studies have examined the effectiveness of propolis extracts against the greater wax moth, an opportunistic parasite that mainly affects weakened hives (Johnson et al. 1994; Garedew et al. 2004). In laboratory experiments similar to those conducted with *Varroa*, propolis extracts caused larval mortality and reduced metabolic rates of wax moth larvae and adults (Garedew et al. 2004). The implication here is that contact or possibly volatile emissions from propolis may reduce the ability of the moths to effectively reproduce and develop within a hive. With respect to other large invaders, Cape honeybees, *A. m. capensis*, have been observed encapsulating the parasitic small hive beetle, *Aethina tumida*, in "propolis prisons" which serves to prevent the beetles from successfully reproducing (Neumann et al. 2001). The bees could readily detect the beetles which they chased and tried to bite. Occasionally, a beetle was killed outright by the bees, who cut off its head. But a beetle attacked by worker bees was usually unharmed because it could simply retract its head and legs into its tough shell, similar to a tortoise. The beetles often hide in cracks in the hive, but this is where their luck often runs out. The worker bees encapsulate the beetles in a propolis prison. In short, the beetle is surrounded in propolis resin deposited by the bees, and starves to death. During the building of the prison, when the walls are

not complete, the open end is guarded by one or a few bees who prevent the beetle from escaping. Careful inspections of normal bee hives' uncovered prisons and dead beetles showed that it is a normal defense mechanism, not just something that occurred only in observation hives. This encapsulation defense is effective provided that the bee colony is reasonably strong and the beetles not too numerous. However, if the beetle numbers get out of hand, the bees have another defense. They simply abscond. That is, they rear out their brood and fly away in a swarm to nest elsewhere. In doing this, they leave the beetles behind. Absconding is very rare in European bees but is very common in African and Asian honeybees. Absconding is not a very good option for European bees because they cannot take their food stores with them, and would likely starve to death the next winter. However, in tropical and subtropical areas, food stores are not so vital because foraging can go on year round.

The European honeybee, *A. mellifera*, will also embalm other intruders that are presumably too large to remove from the nest after being killed; Hoyt (1965) observed a mouse encased in propolis and suggested that the bees covered it in propolis to prevent odour and decay from affecting the rest of the hive. Colonies of *A. dorsata* have also been noted to coat foreign objects in propolis (Seeley and Morse 1976), as have the stingless bee *Trigona carbonaria* that “mummify” beetle parasites alive using a mixture of wax, plant resins and mud (also known as batumen; Greco et al. 2009). It may be that this behaviour of embalming predators or parasites may be a relatively widespread phenomenon among the social bees. Particularly with respect to this entombment behaviour, the use of propolis by bees can be described analogously to individual immune function. If we consider a honeybee colony as one entity or “superorganism”, then this behaviour would be equivalent to cellular encapsulation of foreign microbes or parasites seen at the individual level (Cremer and Sixt 2009). The propolis envelope itself also fits into this analogy as it is a type of mechanical barrier to both reduce parasites from entering the nest (or superorganism) and potentially prevents parasites and microbes from developing once inside (Simone et al. 2009).

20.11 Nest Defense by *Apis florea*

Apis florea Fabricius is widespread throughout Asia, where it builds its single comb around small branches of bushes and shrubs (Akratanakul 1977). The exposed position of the comb with the honey storage, bees and brood attracts a range of predatory species, so it is not surprising that *A. florea* possesses a wide array of specific social defense mechanisms among which shimmering (Butler 1954; Pirk et al. 2002) and hissing are most conspicuous. Shimmering behaviour is released by optical stimuli and for example a butterfly—apparently attracted by the odour of honey—releases “shimmering behaviour” (a specific movement) of a few bees hanging in the curtain and which otherwise does not interfere with colony activities. Hissing behaviour is released by mechanical stimuli or by “piping” of forager bees disturbed on their return to the nest (Sen Sarma et al. 2002). Hissing alerts the whole colony and results

in steep decrease of foraging activities. The hissing sound per se may repel mammals and even large Asian bears (Koeniger and Fuchs 1973) or initiate a full stinging defense of the colony (Koeniger and Fuchs 1975).

A different permanent threat in the arboreal habitat of *A. florea* is posed by the weaver ant *Oecophylla smaragdina* (Hölldobler and Wilson 1983), which ranks among the most important predators of *A. florea* (Seeley 1983). In direct fighting, the *A. florea* workers, because of their diminutive size, are no match for the large weaver ants. Colonies rely instead on sticky bands of plant resins plastered around the branch carrying the nest. Thus the comb with the brood, the honey storage and the bees are sealed off completely by sticky barriers on all structures connecting to other parts of the canopy. Duangphakdee et al. (2005) reported that in the arboreal habitat of *Apis florea*, one of the dominant insectivorous predators is the weaver ant, *Oecophylla smaragdina*. The main mechanism of *A. florea* to protect its nest against ants and other crawling arthropods are “barriers” of sticky material (sticky bands) which the bees build around the branches and all structures which connect the comb to the outside.

The reinforcement of the sticky barriers seems to be a specific behavioural response of *A. florea* to its most prominent predator *O. smaragdina*. A comparable phenomenon termed enemy specification (Wilson 1975) is known to operate between several ant species. These species recognize their most dangerous adversary species and fight against them without delay by specific mass attacks (Hölldobler 1983). The question of how *A. florea* recognizes the weaver ant remains open. The experiments, however, with *C. rogenhoferi* and larvae of *Tenebrio* point to possible cues or semiochemicals specific to *O. smaragdina*.

20.12 Mechanical and Chemical Weapons for Brood Protection in Social Insects

Chemical weapons and defense secretions are additional features inflicting pain or death on intruders, acting as allomones against predators or as fungicides and bactericides against parasites (Blum 1981). Schmidt (1990) has suggested that, without venoms, social Hymenoptera would probably not exist due to the almost insurmountable problem of vertebrate predation (Table 20.3). Social insect colonies are targets for invertebrates and vertebrates acting as parasites and predators. Various pathogen and parasite groups of social insects such as viruses, bacteria, fungi, nematodes and mites have been recently reviewed by Schmid-Hempel (1998). However, brood is also endangered by conspecific individuals of other nests that may enter a colony and steal brood. Intraspecific brood parasitism is found in all groups of social Hymenoptera (Wilson 1971) and, even within a colony, conflicts over reproduction may arise amongst nestmates and influence the survival of eggs and larvae. The honeybees *Apis mellifera* and *A. cerana* usually nest within enclosed cavities, presumably as a defense against powerful predators such as honey badgers and bears (Seeley 1983).

Table 20.3 Mechanisms of brood protection in social insects

(A) Mechanical defense.								
Taxon	Appro- priate	Camou- flage	Entrance turrets pedicels	Closure of nest entrance, burrow plugs	Cell walls, cell cups	Abdom- inal burst- ing	Guards	Grooming, hygienic behaviour
Ants	x			x ^a		x	x ^b	x
Bees	x	x	x	x	x		x	x
Wasps	x	x	x	x	x		x	x
Termites	x			x ^a		x	x ^b	x
(B) Chemical defense								
Taxon	Chemical barriers	Alarm pheromones			Chemical weapons defense secre- tions	Antiseptic chemicals		
Ants	x		x		x ^c			x ^d
Bees	x		x		x ^c			x ^d
Wasps	x		x		x ^d			x
Termites	x		x		x			x

^aphragmotic heads
^bmorphological soldier castes
^cbite
^dsting

20.12.1 Alarm Pheromones

Alarm behaviour mostly occurs as a result of an external threat to a colony or as a result of a signal (chemical or otherwise) produced by nestmates (Landolt et al. 1998). Therefore, the most important role of alarm behaviour is to protect the colony and its brood from predators. Alarm pheromones occur in all groups of social insects and are employed in or near the nest.

In honeybees, alarm pheromones are produced primarily by cells of the setose membrane surrounding the sting shaft base (Ghent and Gary 1962; Maschwitz 1964). Chemical analysis of the volatile compounds extracted from the sting apparatus of guarding workers reveals about 70 % isopentyl acetate, with the remainder a complex blend of mostly C4 to C10 alcohols and their acetates (Boch et al. 1962; Boch and Shearer 1967; Blum et al. 1978; Collins and Blum 1982a, b; Schmidt 1998). The mandibular glands in the honeybee worker's head produce 2-heptanone, a compound that is at least 20 times less potent than isopentyl acetate in signalling alarm (Shearer and Boch 1965; Boch et al. 1970). The alarm pheromone is released from autotomized stings imbedded into attacked animals, thereby creating a chemical signal which guides other guards to the enemy (Seeley 1983). Alarm pheromones of honeybees and stingless bees can mobilise masses of individuals and orchestrate probably the best insect defense on earth (Roubik 1989; Schmidt 1998).

20.13 Conclusions

It is clear that social insects possess a variety of defense mechanisms to protect their colony and its brood from interspecific attack by a wide range of pests, predators and pathogens (Schmid-Hempel 1998). Many of these mechanisms are underpinned, directly or indirectly, by odours used variously as pheromones, allomones and kairomones; indeed, social insect workers are superlative chemists, utilising both self-synthesized and environmentally acquired compounds in defense (Blum 1981). There are clearly a multitude of options for future research related to propolis and resin use by bees ranging from the pharmacological opportunities for human health to understanding the individual and colony-level mechanisms of resin foraging to the possible applicability for propolis as a treatment against bee pathogens and diseases. At the least, information on resin use and its incorporation into the honeybee nest architecture is an exciting area of research concerning environmental impacts on disease resistance and social immunity. Our contention is that a better understanding of proximate mechanisms will provide a more comprehensive account of the role of kin selection in shaping social insect societies and, at the same time, make possible manipulative experiments that examine the ultimate factors shaping reproductive conflicts.

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Chapter 21

Ecological Impacts of Invasive Alien Species on Bees

21.1 Introduction

The devastating impacts that some exotic organisms have wreaked on native ecosystems surely ought to have taught us a lesson as to the perils of allowing release of alien species. The introduction of Nile perch to Lake Victoria, and of cane toads, prickly pear, rabbits, foxes, and cats among numerous others to Australia, are perhaps some of the best known examples, but they constitute only the tip of the iceberg. Australia alone had 24 introduced mammal species, 26 birds, 6 reptiles, 1 amphibian, 31 fish, more than 200 known invertebrates, and no less than 2,700 non-native plants at the last count (Alexander 1996; reviewed in Low 1999). A strong case can be made that exotic species represent the biggest threat to global biodiversity after habitat loss (Pimm et al. 1995; Low 1999). Although the threat posed by exotic species is now widely appreciated, exotic bees appear to have received disproportionately little attention. Bees are widely perceived to be beneficial for their role in the pollination of crops and wildflowers and, in the case of the honeybee *Apis mellifera* (L.) (Apidae), for the production of honey. Because of these economic benefits there is reluctance to regard bees as potentially damaging to the environment. In 1872, Darwin stated that honeybees in Australia were “rapidly exterminating the small, stingless native bee”. In fact, the bee he refers to, presumably *Trigona carbonaria* Sm., is still abundant (Darwin 1872). However, almost no research was carried out upon the impact of honeybees until the 1980s, by which time they had long since become established on every continent except Antarctica.

The information provided later reviews the scale on which bees have been artificially distributed around the globe. Three bee species, the honeybee *A. mellifera*, the bumblebee *Bombus terrestris* (L.) (Apidae), and the alfalfa leafcutter bee *Megachile rotundata* (Fabr.), are of particular concern because their range has been considerably expanded owing to both deliberate and accidental releases.

The possible undesirable effects of exotic bees include the following:

1. Competition with native flower visitors for floral resources;
2. Competition with native organisms for nest sites;
3. Transmission of parasites or pathogens to native organisms;

4. Changes in seed set of native plants (either increases or decreases);
5. Pollination of exotic weeds.

I will examine evidence for each of these processes in turn. Reviewing studies to date serves to highlight the substantial gaps in our knowledge. I suggest further experimental approaches that may provide less equivocal answers as to the threat posed by these exotic organisms.

21.2 Distribution and Abundance of Introduced Bees

The honeybee is thought to be native to Africa, western Asia, and southeast Europe (Michener 1974), although its association with man is so ancient that it is hard to be certain of its origins. It has certainly been domesticated for at least 4,000 years (Crane 1990a, b) and has been introduced to almost every country in the world. It is now among the most widespread and abundant insects on earth. The European strain of the honeybee appears to be adapted to temperate and Mediterranean climates, and flourishing feral populations occur throughout much of Asia, North America, the southern half of South America, and Australia. Major events in this range expansion include its introduction to North America in about 1620 (Buchmann and Nabhan 1996), to Australia in 1826 (Doull 1973), and to New Zealand in 1839 (Hopkins 1911). The African race, *A. mellifera scutellata* Lepeletier, is associated with tropical forests and savannas, and has spread throughout the neotropics and into North America following its introduction to Brazil in 1957.

More recently, bumblebees (*Bombus* spp.), a group whose natural range is largely confined to the temperate northern hemisphere, have been introduced to various countries to enhance crop pollination. New Zealand has four established *Bombus* species native to the UK, *B. hortorum* (L.), *B. terrestris*, *B. subterraneus* (L.), and *B. ruderatus* (F.), following introductions in 1885 and 1906 intended to improve pollination of red clover, *Trifolium repens* (Hopkins 1914). *B. hortorum* and *B. subterraneus* have restricted distributions within New Zealand, whereas *B. terrestris* and *B. ruderatus* have become ubiquitous (Macfarlane and Gurr 1995). *B. terrestris* spread into Israel in the 1960s (Dafni and Shmida 1996), perhaps as a result of the presence of introduced weeds. This species has also become established in the wild in Japan following escapes from commercial colonies used for pollination in glass houses (Dafni 1998). Most recently, *B. terrestris* arrived in Hobart, Tasmania, in 1992, perhaps accidentally transported in cargo, and has since spread out to occupy a substantial portion of the island (Buttermore 1997; Stout and Goulson 2000; Hingston et al. 2002). *B. ruderatus* was introduced to Chile in 1982 and 1983 for pollination of red clover (Arretz and Macfarlane 1986), and by 1994, it had spread to Argentina (Abrahamovich et al. 2001).

The only other group of bees to have been deliberately redistributed around the globe in substantial numbers are the Megachilidae. Perhaps because of the importance of alfalfa as a crop in the USA, a plant which is not adequately pollinated by honeybees, this country has shown particular enthusiasm for introducing exotic

pollinators. The most widespread is *M. rotundata*, a native of Eurasia that appeared in North America in the 1930s, and which is now widely used commercially for pollination of alfalfa (Bohart 1972). A range of other species have been imported to pollinate various crops, including *Osmia cornuta* Latr. from Spain for pollination of almonds (Torchio 1987), *Osmia cornifrons* (Radoszkowski) from Japan for pollination of fruit trees (Batra 1979), *Osmia coerulescens* (L.) from Europe for pollination of red clover (Parker 1981), and *Megachile apicalis* Spinola from Europe for pollination of alfalfa (Cooper 1984; Stephen 1987). Furthermore, species have been moved within the USA and established in regions far from their home ranges; *Osmia ribifloris biedermannii* Michener from the west coast has been released in Maine to pollinate blueberries (Stubbs et al. 1994). At least three exotic Megachilidae are now established in California, *M. apicalis*, *M. rotundata*, and *M. concinna* (Smith) (Frankie et al. 1998), and *M. rotundata* even occurs in the Everglades National Park, Florida (Pascarella et al. 1999). New introductions continue to occur; for example, *M. sculpturalis* Smith, a native of China and Japan was recently recorded in North Carolina (Mangum and Brooks 1997).

The fate of some deliberate introductions is not known. For example, *Chalicodoma nigripes* from Egypt and *Pithitis smaragdula* F. from India were introduced to the USA in the 1970s for pollination of alfalfa, but to my knowledge, it is not known if these species became established (Daly et al. 1971; Parker et al. 1976). One megachilid, *Megachile rotunda*, was introduced to New Zealand in 1971 for pollination of alfalfa and flourished (Donovan 1975). Recently, this species also became established in southern Australia (Woodward 1996). One final bee that has expanded its range with the deliberate help of man is the alkali bee, *Nomia melanderi* (Cockerell) (Halictidae). This native of North America was introduced to New Zealand in 1971 for pollination of alfalfa and has become established at restricted sites (Donovan 1975, 1979). A summary of the distribution of exotic bee species is given in Table 21.1.

Bees are a large group (about 20,000 species are known), but little is known about basic aspects of the ecology of most species. For the majority we have only a rudimentary knowledge of their natural distribution. It is almost certain that other species have been transported by man to new locations, but that these events have gone unrecorded.

Both *B. terrestris* and *A. mellifera* are social species, with colonies attaining sizes of up to 500 and 50,000 individuals, respectively. In their natural range, nest density estimates for *A. mellifera* vary from 0.5 to > 70 nests/km² in Europe (Visscher and Seeley 1982; Oldroyd et al. 1995) and 4.2 nests/km² in Botswana (McNally and Schneider 1996). Where honeybees have been introduced, estimates include 50–150 nests/km² in southern Australia (Oldroyd et al. 1997) and 6–100 nests/km² for Africanized bees in the neotropics (Roubik 1983, 1988; Otis 1991). Densities are no doubt greatly influenced by variation in habitat quality and availability of nest sites. Given the large numbers of workers per nest, even the lowest estimates indicate substantial densities of foragers. No information is available on densities of nests of *B. terrestris*, either within their natural range or where they are introduced, because they are notoriously hard to locate.

Table 21.1 The distribution and origins of known exotic bee species

Species	Family	Introduced range	Origin	References
<i>A. mellifera</i>	Apidae	North and South America, eastern Asia, Australia, and New Zealand	Eastern Europe, western Asia, Africa	Hopkins 1911, Doull 1973, Buchmann and Nabhan 1996
<i>A. mellifera</i>	Apidae	India	Italy	Atwal 1962
<i>B. terrestris</i>	Apidae	Israel, Japan, New Zealand, Tasmania, Europe	Europe	Hopkins 1914, Dafni and Shmida 1996, Buttermore 1997, Dafni 1998, Stout and Goulson 2000
<i>Bombus ruderatus</i>	Apidae	New Zealand, Chile, Argentina	Europe	Hopkins 1914, Arretz and Macfarlane 1986
<i>Bombus hortorum</i>	Apidae	New Zealand, Iceland	Europe	Hopkins 1914, Prys-Jones et al. 1981
<i>Bombus lucorum</i>	Apidae	Iceland	Europe	Prys-Jones et al. 1981
<i>Bombus subterraneus</i>	Apidae	New Zealand	Europe	Hopkins 1914
<i>M. rotundata</i>	Megachilidae	North America, Australia, New Zealand	Eurasia	Bohart 1972, Donovan 1975, Woodward 1996, Frankie et al. 1998, Pascarella et al. 1999
<i>Megachile apicalis</i>	Megachilidae	USA	Europe	Cooper 1984, Stephen 1987
<i>Megachile concinna</i>	Megachilidae	California	Europe	Frankie et al. 1998
<i>Megachile sculpturalis</i>	Megachilidae	North Carolina	China, Japan	Mangum and Brooks 1997
<i>Osmia coerulescens</i>	Megachilidae	USA	Europe	Parker 1981
<i>Osmia cornifrons</i>	Megachilidae	USA	Japan	Batra 1979
<i>Osmia cornuta</i>	Megachilidae	USA	Europe	Torchio 1987
<i>Osmia ribifloris biedermannii</i>	Megachilidae	Maine Southwestern	USA	Stubbs et al. 1994
<i>Pithitis smaragdula</i>	Megachilidae	USA	India	Daly et al. 1971
<i>Chalicodoma nigripes</i>	Megachilidae	USA (establishment unknown)	Egypt	Parker et al. 1976
<i>Nomia melanderi</i>	Halictidae	New Zealand	North America	Donovan 1975, 1979

In general, both honeybees and *B. terrestris* appear to maintain higher population densities than semisocial and solitary species across a broad range of habitats and geographic regions (South Australia, Pyke and Balzer 1985; California, Dobson 1993; Brazil, Wilms et al. 1997; New Zealand, Donovan 1980; Israel, Dafni 1998). It is often impossible to determine how large the equilibrium feral population of honeybees would be because wild populations are supplemented by swarms from commercial hives, and foragers observed in the field are likely to originate from both managed and wild colonies. Little information is available on populations of the introduced Megachilidae, but one study suggests that these solitary species do not attain high densities in Australia (Woodward 1996).

As introduced bees are widespread, any deleterious effects of their presence are now occurring on a large scale. The abundance of honeybees and bumblebees makes such effects more probable. Some researchers have concluded that competition with native organisms is inevitable (Roubik 1978; Roubik and Buchmann 1984; Sugden et al. 1996) in the temperate climates where they naturally occur. Thus, in terms of the time of year at which they are active, they overlap with almost all other flower visitors with which they co-occur.

Although competition from honeybees can affect all nectar-feeding animals, native bees are the logical focus for any investigation. Like honeybees, native bees rely on nectar and pollen for nutrition and for their brood. In addition, native bees are the most dominant pollinators in natural ecosystems and thus are vital to the maintenance of biological diversity (Sugden et al. 1996).

Worldwide, most research to date into honeybee/native bee competition has concentrated on one or more of the following three measurements: the overlap in resource use between honeybees and native bees; the change in visitation rates of native bees; and the change in the levels of resource harvested by native bees when honeybees are present (Table 21.1). The implication of any measurable changes in the above three aspects has been that the presence of honeybees will impact on the fecundity or adult survival of native bees and, ultimately, their population density (Paton 1996). However, such conclusions may not be justified. For competition to occur between honeybees and native bees, there must first be an overlap of floral resource, with both species collecting nectar and pollen from the same flower species (Fig. 21.1). Although both species may visit flowers, competition can be absent if the presence of honeybees fails to interfere with native bee visitation rates or if floral resources are not limiting. Visitation rate and the level of resource harvesting of native bees will, under these conditions, remain unchanged. Even if native bees are experiencing competition from honeybees, they may not be able to change visitation rates in response and the amount of resource harvested will be reduced (Fig. 21.1). Alternatively, the presence of honeybees visiting the same floral resources may cause a decrease in native bee visitation rates. However, as with floral resource overlap, reduced visitation rates of native bees may not necessarily equate to a negative impact. If native bees compensate for reduced visitation rates, for example, by foraging longer through the day, their level of resource harvesting could remain unchanged (Fig. 21.1). Furthermore, if reduced visitation rates of native bees result in a decrease in harvested resource, native bees may use an alternative floral species. If this alternative floral

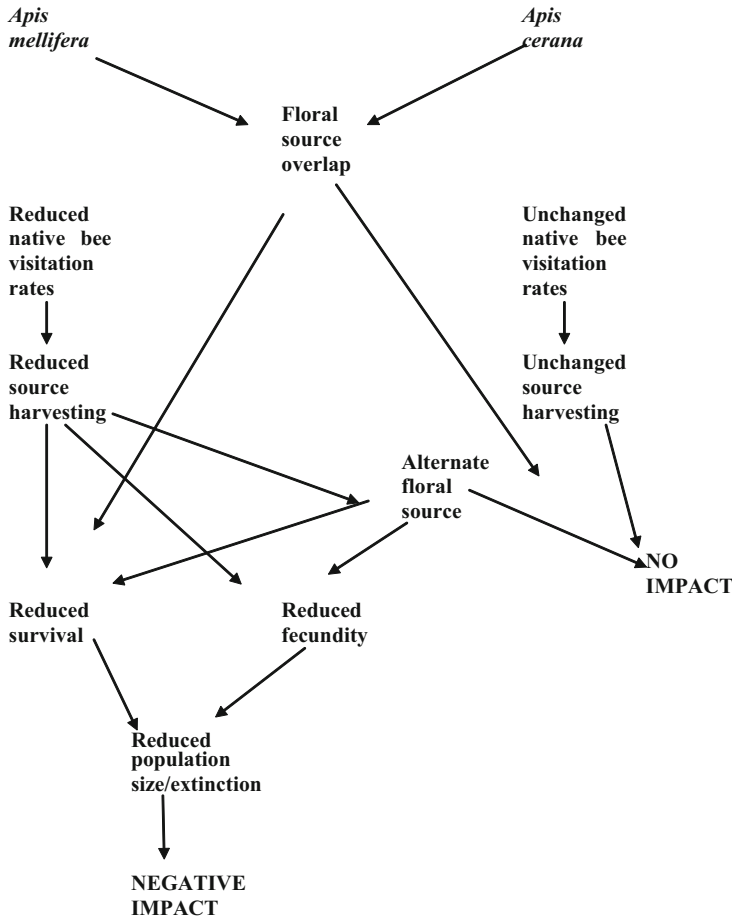


Fig. 21.1 To determine if competition is occurring between honeybees and native bees, researchers have assessed a number of measurements. However, floral source overlap reduced native bee visitation rates and reduced source harvesting can result in either no impact or a negative impact on native bees. Only reduced survival, reduced fecundity, and reduced population size/extinction will definitely lead to a negative impact on native bees. (Paini 2004)

resource provides nectar and pollen in the same quantity and quality at no cost to survival or fecundity, then, although there may be evidence of competition for one resource, there may be no evidence of a negative impact on native bees (Fig. 21.1). Competition can be defined as a negative interaction between populations (Grover 1997), and therefore, any interaction that simply changes the behaviour of native bees can be defined as competition and subsequently a negative impact. This review defines a negative impact as that which reduces individual fitness and therefore threatens the long-term survival of a population. Under this more specific definition, a change in behaviour, such as reduced visitation rate or switching to an alternative

resource, may not lead to a negative impact on the species directly competing with honeybees. Furthermore, any change in native bee behaviour can have implications for the ecosystem it occupies. A native bee that switches from one plant species to another in response to the presence of honeybees may influence pollination of both the new and old plant species, or create a situation where other native pollinators now compete with the native bee species. However, although second- and third-order impacts of this nature can occur, they have never been documented in studies of honeybee/native bee competition. Clearly, the three measurements by which competition between honeybees and native bees can be assessed (floral resource overlap, visitation rates, and resource harvesting) are indirect and may not result in a negative impact on native bees. Only by assessing direct measurements, such as individual survival, fecundity, or population numbers, can a negative impact be determined (Fig. 21.1). Nevertheless, studies investigating these indirect measurements of competition are valuable, as they indicate the potential for competition between honeybees and native bees. Paini (2004) reviewed 28 studies investigating interactions between honeybees and native bees using indirect measurements. In an exhaustive review, Paini 2004 evaluates research on all measurements in which competition between honeybees and native bees occur, focusing on the problems associated with these studies. One of the common flaws in studies investigating the impact of honeybees on native bees has been lack of replication of sites. For example, Pedro and Camargo (1991) collected bees visiting flowers from 140 plant species over a 1-year period in a natural “cerrado” ecosystem in Brazil. Honeybees’ floral preference overlapped with that of native bees, particularly those belonging to the Meliponinae, but sampling occurred at only one site. In the USA, Ginsberg (1983), using information from only one site, found that honeybees foraged on large flower clusters and native bees foraged on small flower clusters. Finally, Bailey (1994) reported an increase in native bee densities when honeybees were prevented from visiting one flowering bush of *Leucopogon propinquus* over a series of days.

The confounding factors compromised the interpretation of results in three (11 %) of the 28 studies. Wratt (1968) compared the visitation rates of two species of bumblebees (*B. terrestris* and *B. ruderatus*) with those of honeybees on red clover (*Trifolium pratense*). As temperature increased, the number of *B. ruderatus* decreased and the number of honeybees increased. Wratt (1968) concluded that competition was occurring between honeybees and *B. ruderatus*. However, an alternative hypothesis, not considered in this study, is that temperature was the factor causing changes in visitation rates. On the Bonin (Ogasawara) Islands in the Pacific Ocean, Kato (1992) and Kato et al. (1999) demonstrated an overlap in resource use between honeybees and a number of native bees including colletids, megachilids, and anthophorids. These authors argued that native bee species had disappeared from two of the islands because of the presence of honeybees. However, the islands have also undergone major habitat destruction and invasion by exotic plants as a result of human activity, and it is equally possible that these factors, rather than honeybees, resulted in a reduction in native bee biodiversity.

Pyke and Balzer (1985) investigated visitation rates of native bees in response to honeybees in four experiments and found conflicting results. In the first experiment,

when honeybees were removed from sites, the number of native bees visiting these sites appeared to increase significantly. In the second experiment, a census area was surveyed at three distances from an established apiary. The authors expected honeybee density to decrease with distance from hives and native bee density to increase, but both honeybee and native bee density increased with distance. In the third experiment, 30 hives were added to a site and honeybees, counted from transects, increased at all plots except the furthest one from the hives (1,000 m). Native bee density decreased at all plots except the furthest, suggesting a negative impact. However, although honeybee visitation rates to flowering *Prostanthera cuneata* (its only nectar source) generally increased when honeybee hives were added, native bee visitations did not decrease significantly. A fourth experiment showed no significant negative relationship between densities of honeybees and native bees on any flowering species. In fact, for *Angophora hispida*, as honeybee density increased so did native bee densities. Despite the contradictory results of the four experiments, the authors concluded that native bees were excluded from or avoided areas of high honeybee densities. Overall, only the first experiment provides any supporting evidence that honeybees negatively impact on native bees, whereas the remaining experiments remain ambiguous.

In the four studies (14 % of studies), the authors recognized the shortcomings of their work and reached few conclusions for a number of reasons. Wilms et al. (1996) calculated the degree of resource overlap for 17 species of native stingless bees and honeybees in Brazil. They found honeybees overlapped more with other stingless bees than stingless bees did with each other, and concluded that stingless bees suffer more competitive pressure from the honeybee than from each other. However, no conclusions were made regarding the possible decline of stingless bees as a result of this competition. In fact, the authors argued that stingless bees should be able to avoid competition with honeybees as the mass flowering conditions that occur during the period in which the experiment was conducted would probably prevent depletion of nectar resources. Again, in South America, honeybees were found to be more common than native bees in small patches of subtropical forest in Argentina, whereas native bees were more common than honeybees in large patches (Aizen and Feinsinger 1994a, b). This result could have been interpreted as evidence for the competitive exclusion of native bees by honeybees, but the complicating factor of patch size prevented the authors from drawing any conclusions. In an Australian study, 70 % of the plants visited by honeybees were also visited by native bees (Wills et al. 1990) and a potential for competition between honeybees and native bees was considered, but again no conclusions were drawn from this study. Gross (2001) found that visitation rates of honeybees and native bees on *Dillwynia juniperina* were negatively related, and this implied a negative impact of honeybees on native bees. However, the author indicated no conclusion could be made without examining brood levels in response to honeybee competition.

When visitation rates are measured, not only exploitative competition can occur but also interference competition, with honeybees displacing native bees from floral resources by their physical presence. For example, in Japan, Sakagami (1959) found that at an artificial nectar source, *A. mellifera* and *Apis cerana* attacked each other

and eventually *A. cerana* were excluded. Roubik (1980) also reported low levels of honeybee aggression towards both meliponine and polybiine wasps at artificial feeders, although he pointed out that aggressive interactions are much more likely with such feeders as they provide unusually rewarding resources. In Australia, Gross and Mackay (1998) found that in 91 % of honeybee/native bee interactions on *Melastoma affine*, native bee foraging was disrupted by honeybees. They also observed honeybees grappling with native bees in an attempt to pull them off flowers.

Finally, three of the 28 studies (11 %) failed to find any impact on native bee visitation rates in response to honeybees. Roubik (1996a, b) followed up an investigation from 17 years earlier (Roubik 1978) in which he had predicted that competition from honeybees may lead to a population decline in native pollinators. There was no evidence for local extinction or population decline of native *Melipona* spp. resulting from honeybee competition. In an Australian study, Paton (1999) found no impact of honeybees on visitation rates of native pollinating insects, and Horskins and Turner (1999) showed that honeybees rarely depleted nectar resources completely.

21.3 Direct Measurements: Survival, Fecundity, and Population Density

Paini (2004) reviewed nine studies that investigated competition by direct measurements such as native bee fecundity, survival or population density (Table 21.1). Three were conducted in Europe (Pechhacker and Zeillinger 1994; Evertz 1995; Steffan-Dewenter and Tscharnkte 2000). Evertz (1995) found that the reproductive success of *M. rotundata* was higher in a site without honeybees than in one with honeybees, but the study lacked site replication (Table 21.1) and other factors could have been involved. The other two European studies found no impact of honeybees on native bees (Pechhacker and Zeillinger 1994; Steffan-Dewenter and Tscharnkte 2000). This is not surprising as honeybees are native to Europe and are likely to have evolved with other native bees to reduce niche overlap and limit competition.

By comparison, in French Guiana, Roubik (1983) introduced colonies of native bees (*Melipona favosa* and *Melipona fulva*) to two sites and then added honeybees for 30 days. There was no evidence of decreased fecundity or resource harvesting of the two *Melipona* spp. while the honeybees were present. In Panama, Roubik and Wolda (2001) recorded bee species caught in two light traps for 7 years before honeybees invaded and 10 years following invasion. There was no evidence of a decrease in the relative population levels of 15 of the most common bee species. Thoenes (1993) found that honeybee hives in the USA attracted native bee species, which were then attacked and killed by the honeybees, suggesting that local populations of native bees could be impacted. As with many other papers researching honeybee/native bee competition, the aforementioned three studies lack replication (two sites, one site, and one site, respectively).

The remaining studies in Table 21.1 were conducted in Australia. Sugden and Pyke (1991) found that honeybees and *Exoneura asimillima*, a native, semisocial

bee in New South Wales, overlapped in their use of nectar and pollen from a wide range of plants. Fewer adult *E. asimillima* were found in established nests at one site with honeybees present than at three sites where they were absent. This was attributed to higher migration or death rates. Additionally, founder nests (newly formed colonies) at the site with honeybees contained more adult females, pupae, and eggs. The authors suggested that adults were being forced out of established nests because resources were depleted by honeybees.

However, Paton (1996) argued that higher numbers of newly formed nests in the presence of honeybees could indicate greater native bee fecundity. But the authors acknowledged that unmeasured aspects of microclimate may have varied between sites with and without honeybees, and without adequate replication, their conclusions were tentative.

Schwarz et al. (1991, 1992a, b) found no negative impact of either commercial or feral honeybees on *Exoneura bicolor* and *Exoneura nigrihirta* in terms of brood mass, brood number, and adult number. Native bee colonies had higher survival at sites where honeybees were present, perhaps because high honeybee numbers can saturate a predator population and reduce predation on native bees. Spessa (1999) investigated the impact of honeybees on a native colletid bee (*Amphylaeus morosus*) at eight sites over two seasons, reversing the control and impact sites between seasons to control for site effects. Although resource overlap between honeybees and *A. morosus* averaged 52 %, no negative impact of honeybee presence could be detected, and in one season, there were more new nests of the native bee in the presence of honeybees. Spessa (1999) concluded that nectar and pollen are not limiting for *A. morosus*.

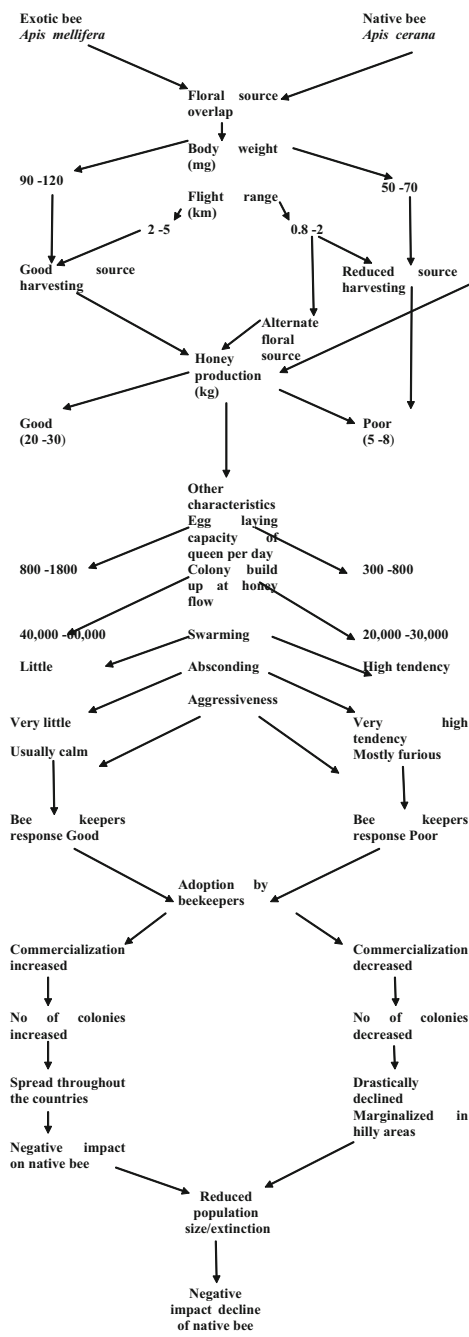
A. mellifera colonies have superior morphometric characteristics and are generally more productive than those of *A. cerana*. Colonies of *A. cerana* with lower productivity and absconding behaviour creates a major obstacle to the development of beekeeping with this bee in rural areas in southern Asia. There is one disadvantage with *A. cerana*: their radius of foraging activity is less than half of that of *A. mellifera*. This means that a colony covers only a quarter of the area covered by *A. mellifera*, and therefore, one colony of *A. cerana* produces one quarter of the honey produced by an *A. mellifera* colony. Because of its aggressive behaviour and high swarming and absconding tendency, it is less preferred by the farmers for commercial beekeeping, resulting in its marginalization and consequently decline in populations (Fig. 21.2)

21.4 Competition with Native Organisms for Floral Resources

Demonstration of niche overlap does not prove that competition is occurring. In fact, it is notoriously difficult to provide unambiguous evidence of competition, particularly in mobile organisms. Thus, there is no clear agreement as to whether non-native bees have had a significant negative impact upon native pollinator populations (compare Robertson et al. 1989, Buchmann and Nabhan 1996, and Sugden et al. 1996 with Butz Huryn 1997).

The majority of studies to date have been carried out in the neotropics, stimulated by the arrival of Africanized honeybees, and in Australia, where awareness of the

Fig. 21.2 Determination of whether competition is occurring between exotic honeybees and native bees. *A. cerana* population has declined because of competition for resources from *A. mellifera* and low adoption by the beekeepers for more honey production and favourable characteristics of *A. mellifera* over *A. cerana*



possible impacts of introduced species is unusually high. Australia also has a large native bee fauna of over 1,500 species (Cardale 1993) that is arguably the most distinctive in the world (Michener 1965). Most work has focused on the effects of honeybees.

21.5 Effects on Foraging Behaviour

Each honeybee nest harvests 10–60 kg/year of pollen and also requires 20–150 kg/year of honey (Stanley and Liskens 1974; Roubik et al. 1984; Buchmann 1996). Crude extrapolation from the range of nest densities that have been recorded suggests that honeybees may gather 5,000–9,000 kg pollen and 10,000–22,500 kg/km²/year honey. In New Zealand, 8,000 tons of honey is harvested from about 227,000 commercial hives every year (Donovan 1980). No direct estimates of the total amounts of pollen or nectar available in natural habitats over a year are available, and there is no doubt that it varies enormously. However, common sense suggests that honeybees must use a substantial proportion of the available floral resources.

Honeybees commonly deter other bee species from foraging on the richest sources of forage (Wratt 1968; Eickwort and Ginsberg 1980; Roubik 1978, 1980, 1996a; Wilms and Wiechers 1997; Gross 2001) (although in one instance, the converse had been reported, Menke 1954). Native organisms are often displaced to less profitable forage (Holmes 1964; Schaffer et al. 1979, 1983; Ginsberg 1983). In Panama, the presence of Africanized honeybees effectively eliminated foraging peaks of *Meliponine* bees because these native species were prevented from visiting their preferred sources of forage; as a result, the rate at which pollen was accrued in the nest was lower (Roubik et al. 1986). Displacement of native organisms has been attributed to the larger size of honeybee when compared with the majority of bee species (Roubik 1980), but is not necessarily size related. For example, the presence of honeybees has been found to deter foraging by hummingbirds (Schaffer et al. 1983). Similarly, in a year when honeybees were naturally scarce, native bumblebees in Colorado were found to expand their diet breadth to include flowers usually visited mainly by honeybees (Pleasants 1981).

Hingston and McQuillan (1999) examined interactions between bumblebees and native bees in Tasmania and concluded that native bees were deterred from foraging by the presence of bumblebees, perhaps because bumblebees depressed availability of floral resources. Honeybees have been shown to depress availability of nectar and pollen (Paton 1990, 1996; Wills et al. 1990; Horskins and Turner 1999), which may explain why other flower visitors then choose to forage elsewhere. Most authors concur that honeybees are not particularly aggressive to other insects while foraging, so that impacts on other species occur primarily through exploitative competition (Schaffer et al. 1979, 1983; Thorp 1987; Roubik 1991). However, honeybees have been found to displace smaller species from flowers by physical disturbance (Gross and Mackay 1998). Honeybees do attack nests of other honey-storing species to steal the honey, a behaviour that may have contributed to the decline of *A. cerana* in Japan (Sakagami 1959) (Table 21.2).

Table 21.2 Studies investigating the impact of honeybees on native bees, grouped into the measurements employed to determine impact. (Paini 2004)

Country	Study animal	Sites	Impact ^a	Reviewed paper ^b	Author(s)
<i>Resource overlap</i>					
Germany	Solitary Apoidea	2	0	Y	Fotler 1995
Ogasawara Islands	Colletidae, Megachilidae, Anthophoridae	7	—	Y	Kato 1992
Brazil	Native bees	1	0	Y	Pedro and Camargo 1991
Brazil	Native bees	1	—	Y	Wilms et al. 1996
Australia	Native bees	90	—	Y	Wills et al. 1990
<i>Visitation rates</i>					
Argentina	Native insects	4	0	Y	Aizen and Feinsinger 1994a, b
USA	Native bees	1	—	Y	Ginsberg 1983
New Zealand	Native insects	4	—	Y	Murphy and Robertson 2000
USA	<i>Bombus bifarius</i> and <i>Bombus flavifrons</i>	2	—	Y	Pleasant 1981
French Guiana	<i>Melipona</i> spp.	1	—	Y	Roubik 1978
French Guiana	<i>Melipona fulva</i> , <i>Trigona</i> spp.	9	—	Y	Roubik 1980
Costa Rica	<i>Trigona corvina</i>	1	—	Y	Roubik 1981
French Guiana	<i>Melipona</i> spp.	1	0	Y	Roubik 1996a, b
Japan	<i>A. cerana</i>	1	—	Y	Sakagami 1959
USA	<i>Bombus sonorus</i> and <i>Xylocopa arizonensis</i>	6	—	Y	Schaffer et al. 1979
USA	<i>Bombus sonorus</i> and <i>Xylocopa arizonensis</i>	1	—	Y	Schaffer et al. 1983
USA	Native bees	2	—	N	Wenner and Thorp 1994
New Zealand	<i>B. terrestris</i> , <i>B. ruderatus</i>	1	—	Y	Watt 1968
Australia	<i>Nomia</i> sp.	1	—	N	Bailey 1994
Australia	Native bees	1	—	Y	Gross and Mackay 1998
Australia	Native bees	2	0	Y	Gross 2001
Australia	Native insects	2	0	Y	Horskins and Turner 1999
Australia	Native insects	15	0	N	Paton 1999
Australia	Native bees	1	—	N	Pyke and Balzer 1985

Table 21.2 (continued)

Country	Study animal	Sites	Impact ^a	Reviewed paper ^b	Author(s)
<i>Resource harvesting</i>					
Brazil	<i>Melipona bicolor</i> and <i>Melipona quadrifasciata</i>	1	—	Y	Wilms and Wiechers 1997
Panama	<i>Melipona</i> spp., <i>Trigona</i> spp.	1	—	Y	Roubik et al. 1986
India	<i>Apis indica</i>	?	—	N	Atwal and Sharma 1971
<i>Adult survival</i>					
USA	<i>Bombus sonorus</i> and <i>Xylocopa californica</i>	1	—	Y	Thoenes 1993
<i>Fecundity</i>					
Germany	<i>M. rotundata</i>	2	—	Y	Evertz 1995
Austria	Native bees	1	0	Y	Pechhacker and Zeillinger 1994
French Guiana	<i>Melipona favosa</i> and <i>Melipona fulva</i>	2	0	Y	Roubik 1983
Germany	Native bees	15	0	Y	Steffan-Dewenter and Tschamtk 2000
Australia	<i>Exoneura bicolor</i> and <i>Exoneura nigrihirta</i>	6–8	0/+	N	Schwarz et al. 1991, 1992a, b
Australia	<i>Amphylaeus morosus</i>	8	0/+	N	Spessa 1999
Australia	<i>Exoneura asinillima</i>	4	—	Y	Sugden and Pyke 1991
<i>Population density</i>					
Panama	Native bees	1	0	Y	Roubik and Wolda 2001

— negative impact, + positive impact, ? authors did not indicate the number of sites used, 0 no impact, N not reviewed, Y reviewed

^a There is considerable information in theses and reports that are listed here as un-reviewed papers

^b Conclusion of the author(s) as to the impact of honeybees on native bees

Both honeybees and bumblebees begin foraging earlier in the morning than many native bee species (Corbet et al. 1993; Dafni and Shmida 1996; Horskins and Turner 1999). Honeybees are able to achieve this owing to their large size (compared with most bees) and also owing to heat retention within their large nests (Roubik 1989). Bumblebees are able to begin foraging earlier still because of their great size and densely hairy body. It has been argued that depletion of nectar before native bees begin to forage may result in a significant asymmetry in competition in favour of these introduced species (Matthews 1984; Hopper 1987; Anderson 1989; Dafni and Shmida 1996; Schwarz and Hurst 1997).

Asymmetries in competition may also occur because of the ability of honeybees and bumblebees to communicate the availability and/or location of valuable food sources with nest mates, thus improving foraging efficiency (von Frisch 1967; Dornhaus and Chittka 1999) (the majority of bee species are solitary, and each individual must discover the best places to forage by trial and error). Therefore, social species are collectively able to locate new resources more quickly, which again may enable them to gather the bulk of the resources before solitary species arrive (Roubik 1980, 1981; Schwarz and Hurst 1997).

Honeybees and bumblebees appear to be unusual in the distances over which they are capable of foraging. Honeybees are known to forage over 10 km from their nest, on occasion up to 20 km (Seeley 1985; Schwarz and Hurst 1997), and *B. terrestris* up to at least 4 km (Goulson and Stout 2001). Little is known of the foraging range of most other bee species, but those estimates that are available suggest that they are generally lower. For example, *Melipona fasciata* travels up to 2.4 km (Roubik and Aluja 1983) and Trigonini over 1 km (Roubik et al. 1986). Solitary bee species are generally thought to travel only a few hundred meters at most (Schwarz and Hurst 1997).

Managed honeybee hives have further advantages over wild bee species; they are often given supplementary feeds when floral resources are scarce, and they are moved to track changing patterns of floral abundance. In this way populations of honeybees may be elevated far above those that could naturally persist in particular habitats.

Asymmetries in competition may not be stable, because the relative competitive abilities of bee species are likely to vary during the day according to temperature and resource availability, and are likely to vary spatially according to the types of flowers available (Corbet et al. 1995). The main exotic bees are large compared with most of the native species with which they may compete; *B. terrestris* weighs 109–315 mg (Prys-Jones 1982) and *A. mellifera* workers 98 ± 2.8 mg (Corbet et al. 1995). They also have longer tongues than many native species, particularly in Australia where most native species are short tongued (Armstrong 1979). Large bees are at a competitive advantage in cool conditions because of their ability to maintain a body temperature considerably higher than the ambient air temperature.

They can thus forage earlier and later in the day than most smaller bees, and during cooler weather. Bees with longer tongues can also extract nectar from deeper flowers. However, large bees are not always at an advantage. The energetic cost of foraging is approximately proportional to weight (Heinrich 1979). Thus, large bees

burn energy faster. As nectar resources decline, the marginal rate of return will be reached more quickly by large bees. Also, long tongues are inefficient at handling shallow flowers. Thus, large bees are likely to be at a competitive advantage early in the day and during cool weather, and they will be favoured by the presence of deep flowers that provide them with a resource that other bees cannot access. But small bees with short tongues can forage profitably on shallow flowers even when rewards per flower are below the minimum threshold for large bees. Although in general honeybees and bumblebees are able to forage at cooler temperatures than native bees, there may be exceptions. The Australian native *Exoneura xanthoclypeata* is adapted for foraging in cool conditions (Tierney 1994). It has been argued that this species is specialized for foraging on (naturally) uncontested resources early in the day, and that this species may be particularly susceptible to competition with exotic bees that forage at the same time (Schwarz and Hurst 1997).

The outcome of interactions between exotic and native flower visitors depends upon whether floral resources are limiting. Resource availability is likely to vary greatly during the year as different plant species come into flower (Carpenter 1978). When an abundant or large plant flowers, it may provide a nectar flush. Competition is unlikely to occur during such periods (Tepedino and Stanton 1981). Overall, it seems probable that depression of resources by introduced bees is likely to have negative effects on native bee species, at least at some times of the year. To determine whether these effects are largely trivial (such as forcing native bees to modify their foraging preferences) or profound (resulting in competitive exclusion), population-level studies are necessary.

21.6 Evidence for Population-Level Changes

The only way to test unequivocally whether floral resources are limiting is to conduct experiments in which the abundance of the introduced bee species is artificially manipulated, and the population size of native species is then monitored. If populations are significantly higher in the absence of the introduced bee, then competition is occurring. Such experiments have proved to be exceedingly hard to accomplish. Excluding bees from an area is difficult. Within- and between-season variation is likely to be large, so such experiments need to be well replicated, with replicates situated many kilometres apart, and conducted over several years. No such study has been carried out.

An alternative approach, which is far easier but provides more equivocal data, is to correlate patterns of diversity of native bees with abundance of exotic bees, without manipulating their distribution. Aizen and Feinsinger (1994a, b) found that fragmentation of forests in Argentina resulted in a decline in native flower visitors and an increase in honeybee populations. Similarly, Kato et al. (1999) studied oceanic islands in the northwest Pacific and found that indigenous bees were rare or absent on islands where honeybees were numerous. On Mt. Carmel in Israel, Dafni and Shmida (1996) reported declines in abundance of medium- and large-sized native

bees (and also of honeybees) following arrival of *B. terrestris* in 1978. Conversely, Goulson et al. (2002) found no evidence for reduced abundance or diversity of native Tasmanian bees in areas colonized by *B. terrestris*, but did find that native bee abundance was considerably higher in the few sites where honeybees were absent. However, such studies can be criticized on the grounds that the relationship between exotic bee abundance and declining native bee populations (if found) need not be causative (Butz Huryn 1997). Increasing honeybee populations are often associated with increased environmental disturbance by man, which may explain declines in native bees.

Some researchers have attempted to manipulate numbers of introduced bees, either enhancing populations in experimental plots by placing hives within them or conversely by remove hives from experimental plots in areas where hives have traditionally been placed. Areas without hives usually still have some honeybees, since there are likely to be some feral nests, and also because honeybees can forage over great distances. Replicates of the treatment without hives need to be sited many kilometres from replicates with hives to ensure that bees do not travel between the two, so many studies have been carried out without replication (e.g., Sugden and Pyke 1991). Despite these limitations, some interesting results have been obtained. Wenner and Thorp (1994) found that removal of feral nests and hives from part of Santa Cruz Island in California resulted in marked increases in numbers of native bees and other flower-visiting insects. Addition of honeybee hives caused the Australian nectivorous bird *Phylidonyris novaehollandiae* to expand its home foraging range and to avoid parts of inflorescences favoured by honeybees (Paton 1993), but a comparison of areas with and without hives found no difference in the density of this bird species (Paton 1995). Roubik (1978) found a decrease in abundance of native insects when he placed hives of the Africanized honeybee in forests in French Guiana. However, Roubik (1982a, 1983) found no consistent detrimental effects on brood size, honey, and pollen stores in nests of two Meliponine bee species in Panama when Africanized honeybee hives were placed nearby for 30 days.

Monitoring of numbers of native bee species using light traps over many years since the arrival of Africanized bee has not revealed any clear declines in abundance (Wolda and Roubik 1986; Roubik 1991; Roubik and Wolda 2001). Roubik (1996a) describes the introduction of Africanized honeybees to the neotropics as a vast experiment, but it is an experiment without replicates or controls, so interpreting the results is difficult. Sugden and Pyke (1991) and Schwarz et al. (1991, 1992a, b) failed to find clear evidence for a link between abundance of honeybees and reproductive success of anthophorid bees belonging to the genus *Exoneura* in Australia in experiments in which they greatly enhanced honeybee numbers at experimental sites.

However, the native species that they studied are themselves polylectic (Schwarz and Hurst 1997). As such they are the species least likely to be affected by competition. The majority of bee species are more specialized; in a review of data for 960 solitary bee species, Schemske (1983) found that 64 % gathered pollen from only one plant family, often only one genus. For example, some Australian halictine bees have only been recorded on flowers of *Wahlenbergia* sp. (Michener 1965). Very little is known about such species, and no studies have been carried out to determine

whether they are adversely affected by exotic bees (Schwarz and Hurst 1997). Also, the Australian studies of Sugden and Pyke (1991) and Schwarz et al. (1991, 1992a, b) were carried out in flower-rich heath lands; floral resources are more likely to be limiting in arid regions of Australia (Schwarz and Hurst 1997), and these areas often contain the highest native bee diversity (Michener 1979; O'Toole and Raw 1991). The *Exoneura* species studied in Australia had coexisted with honeybees for 180 years, so it is not surprising that they are not greatly affected by competition with this species. If there are species that are excluded by competition with exotic bees, honeybees in particular, there is no point looking for them in places where these bees are abundant. Unfortunately, this leaves rather few places where they may occur. Overall, there is no indisputable evidence that introduced bees have had a substantial impact via competition with native species. Given the difficulties involved in carrying out rigorous manipulative experiments, this should not be interpreted as the absence of competition. The abundance of exotic bees, the high levels of niche overlap, and evidence of resource depression and displacement of native pollinators all point to the likelihood that competition is occurring. However, we do not know whether such competition results (or resulted) in competitive exclusion. The best way to test for such competition is to carry out replicated experiments in which exotic bee numbers are manipulated and native pollinator numbers and reproductive success monitored over long periods. Ideally, such studies should target native species that are not generalists and areas where floral resources are not abundant.

21.7 Competition for Nest Sites

Honeybees nest in cavities, usually in old trees, and there is clear potential for competition. Many other organisms, including bees, mammals, and birds use such cavities for shelter or for nesting. In managed woodland, old trees with cavities are often in short supply. Hence, it seems likely that honeybees may compete with native organisms for these sites, but rigorous studies are scarce. Both Oldroyd et al. (1994) and Moller and Tilley (1989) found that nesting holes were not in limiting supply in particular forests in Victoria and New Zealand, respectively. However, both studies were confined to small geographic areas, and it is hard to draw any general conclusions without further work. *B. terrestris* generally nests in existing cavities below ground, often using abandoned rodent holes (Donovan and Weir 1978), and spaces beneath man-made structures such as garden sheds (personal observation). To my knowledge there have been no studies to determine whether such sites are used by native organisms in any of the countries to which this species has been introduced, although Donovan (1980) considered it unlikely that bumblebees compete with native bee species for nest sites in New Zealand. Megachilidae nest in small cavities in wood. Donovan (1980) reported that nests sites used by *M. rotundata* overlap with those used by native bees belonging to the Hylaeinae, and also with mason wasps and spiders in New Zealand. However, it is not known whether availability of sites is limiting. Barthell and Thorp (1995) found that introduced *M. apicalis* in California

aggressively usurp native species from nests sites, and concluded that competition was likely. However, subsequent work suggested that differences in habitat preferences between native and introduced species, and an abundance of nest sites may mean that competition is weak or absent (Barthell et al. 1998). Nothing is known of niche overlap in nesting requirements between introduced Megachilidae and native species elsewhere in North America or in Australia.

21.8 Transmission of Parasites or Pathogens to Native Organisms

A great deal is known about the pathogens and parasites of honeybees, and to a lesser extent bumblebees and leafcutter bees, since these species are of economic importance. Bees and their nests support a diverse microflora including pathogenic, commensal and mutualistic organisms (Gilliam and Taber 1991; Goerzen 1991; Gilliam 1997). Many pathogens are likely to have been transported to new regions with their hosts, particularly where introductions were made many years ago when awareness of bee natural enemies was low. Thus, for example, the honeybee diseases chalkbrood, caused by the fungus *Ascosphaera apis*, foulbrood, caused by the bacteria *Paenibacillus* larvae, the microsporidian *Nosema apis*, and the mite *Varroa destructor* now occur throughout much of the world. Hive beetles, *Aethina tumida*, were recently transported from Africa to North America, where they are proving to be serious pests of commercial honeybee colonies (Evans et al. 2000). Similarly, bumblebees in New Zealand are host to a parasitic nematode and three mite species, all of which are thought to have come from the UK with the original introduction of bees (Donovan 1980). During more recent deliberate introductions of exotic bees, such as that of *N. melanderi* to New Zealand, care has been taken to eliminate pathogens or parasites before bees were released (Donovan 1979). However, parasites are easily overlooked. Queens of *Bombus ignitus* are currently sent from their native Japan to the Netherlands, where they are induced to found colonies. The colonies are then returned to Japan for commercial purposes. Goka et al. (2001) recently discovered that the returned colonies are infested with a European race of the tracheal mite *Locustacarus buchneri*. It is hard to exaggerate our ignorance of the natural enemies of most bee species, particularly their pathogens. We do not know what species infect them, or what the host ranges of these pathogens are. Very little is known of the susceptibility of native organisms to the parasites and pathogens that have been introduced with exotic bees. In a survey of natural enemies of native and introduced bees in New Zealand, Donovan (1980) concluded that no enemies of introduced bees were attacking native bees, but that the converse was true. A *chalcidoid* parasite of native bees was found to attack *M. rotundata* and, rarely, *B. terrestris*. One fungus, *Bettisia alvei*, which is a pathogen of honeybee hives elsewhere in the world, was recorded infecting a native bee in New Zealand, but it is not known whether the fungus is also native to New Zealand. Indeed, the natural geographic range of bee pathogens is almost wholly unknown. Some bee pathogens have a broad host

range; for example, chalkbrood (*A. apis*), is also known to infect *A. cerana* (Gilliam et al. 1993) and the distantly related *Xylocopa californica* (Gilliam et al. 1994). The related chalkbrood fungus *Ascosphaera aggregata* is commonly found infecting *M. rotundata*; in Canada, where *M. rotundata* is an exotic species, this fungus infects the native bees *Megachile pugnata* Say (Goerzen et al. 1992) and *M. relativa* Cresson (Goerzen et al. 1990).

It seems likely that these few recorded instances of exotic bee pathogens infecting native species are just the tip of the iceberg, since so few studies have been carried out. As to whether these pathogens have had, or are having, a significant impact on native species, we do not know; if the introduction of a new pathogen were to lead to an epizootic in native insects, it would almost certainly go unnoticed. In other better known organisms, exotic pathogens have had disastrous impacts; for example, the introduction of several crayfish species from North America has led to elimination of the native species *Astacus astacus* and *Austropotamobius pallipes* from large portions of Europe. The native species have little resistance to the exotic fungal pathogen *Aphanomyces astaci* that is carried by the introduced crayfish (Butler and Stein 1985). Studies of the incidence and identity of pathogen and parasite infestations of wild populations of native bees are urgently needed.

21.9 Effects on Pollination of Native Flora

Recently, concerns have been expressed that exotic bees may reduce pollination of native plants, or alter the population structure of these plants by mediating different patterns of pollen transfer to native pollinators (Butz Huryn 1997; Gross and Mackay 1998). Efficient pollination requires a match between the morphology of the flower and that of the pollinator (reviews in Ramsey 1988; Burd 1994). If there is a mismatch, then floral rewards may be gathered without efficient transfer of pollen, a process known as floral parasitism (McDade and Kinsman 1980). Specialized obligate relationship between plants and pollinators do exist (reviewed in Goulson 1999) but are the exception (Waser et al. 1996). Most flowers are visited by a range of pollinator species, each of which will provide a different quality of pollinator service. The efficiency of honeybees as pollinators of native plants in Australia and North America was reviewed by Butz Huryn (1997). She concluded that honeybees provide an effective pollination service to the majority of the flower species that they visit, although they do act as floral parasites when visiting a small number of plant species such as *Grevillea X gaudichaudii* in Australia (Taylor and Whelan 1988) and *Impatiens capensis* and *Vaccinium ashei* in North America (Wilson and Thomson 1991; Cane and Payne 1988). Similar results have been found for honeybees visiting Jamaican flora (Percival 1974). That honeybees are effective pollinators of many plants, even ones with which they did not co-evolve is not surprising. After all, they have been used for centuries to pollinate a broad range of crops. Thus, pollination of the native Australian *Banksia ornata* was increased by the presence of honeybee hives (Paton 1995), and honeybees have proved to be as effective as native bees in

pollinating wild cashews, *Anacardium occidentale*, in South America (Freitas and Paxton 1998). However, their presence may result in reduced seed set of some native plants. Roubik (1996b) reported declining seed set in the neotropical plant *Mimosa pudica* when honeybees were the dominant visitors, compared with sites where native bees were the more abundant, whereas Aizen and Feinsinger (1994a, b) found reduced pollination of a range of Argentinian plant species in areas where forests were fragmented and honeybees more abundant. Gross and Mackay (1998) demonstrated that honeybees were poor pollinators of the Australian native *Melastoma affine*, so that when honeybees were the last visitors to a flower, seed set was reduced. As Roubik (1996b) points out, if native pollinators are lost (be it through competition with exotic bees, habitat loss, or use of pesticides) then we cannot expect honeybees to provide an adequate replacement pollination service for all wild plants and crops.

No studies have yet been reported of the effects of exotic bumblebees on the seed set of native plants. *B. terrestris* has the potential to disrupt pollinator services in a different way. This bee species is known to rob flowers. When the structure of the flower renders the nectaries inaccessible, *B. terrestris* (and some other bee species) may use their powerful mandibles to bite through the base of the corolla (Inouye 1983). In this way, they act as floral parasites, removing nectar without effecting pollination. In Tasmania they rob some bird-pollinated plants in this way (personal observation). The effects of this behaviour are hard to predict. Robbers have been found to reduce the amount of reward available, resulting in decreased visitation rates by pollinators (McDade and Kinsman 1980) and a reduction in seed set (Roubik 1982b; Roubik et al. 1985; Irwin and Brody 1999). Robbing can damage floral tissues preventing seed production (Galen 1983). However, nectar robbing may have little influence on plant fecundity if nectar robbers also collect pollen and in doing so effect pollination, or if other pollinators are present (Newton and Hill 1983; Arizmendi et al. 1995; Morris 1996; Stout et al. 2000). Some plants may actually benefit from the activity of nectar robbers by forcing legitimate foragers to make more long-distance flights, hence increasing genetic variability through out-crossing (Zimmerman and Cook 1985).

A second possible detrimental effect of exotic bees is that they may alter the population structure by effecting a different pattern of pollen transport to native pollinators. In South Australia, Paton (1990, 1993) found that honeybees extracted more nectar and pollen from a range of flower species than did birds, the primary native pollinators. However, honeybees moved between plants far less than did birds, and so were less effective in cross-pollination, resulting in decreased seed set. Several other studies have reported that inter-plant movement by honeybees is lower than that of other visitors (McGregor et al. 1959; Heinrich and Raven 1972; Silander and Primack 1978). Of course other pollinators often also move small distances, and it has been argued that honeybees are not unusual in this respect (Butz Huryn 1997). However, this is not true. Workers of social bees are unusual in that they are not constrained in their foraging behaviour by the need to find mates, locate oviposition sites, or guard a territory. In contrast, for example, butterflies intersperse visits to flowers with long patrolling flights in which they search for mates or oviposition sites (Goulson et al. 1997). Thus, honeybees, bumblebees, and other social bees do

tend to engage in fewer long flights than other species (Schmitt 1980; Waser 1982). The most obvious possible effect of exotic social bees in this respect is increased self-pollination, which could result in reduced seed set if the plant is self-infertile. Reduced inter-patch pollen movement could result in reproductive fragmentation of plant populations. There are at present no data available on the impact of exotic bees on the genetic structure of plant populations. Clearly, it is not possible to generalize as to the effects that exotic bees will have on seed set of native flowers. For some species, they will provide effective pollination, for others, they will not. Where native pollinators have declined for other reasons, for example, as a result of habitat loss and fragmentation, exotic bees may provide a valuable replacement pollinator service for native flowers. Where exotic bees are floral parasites, the effect will depend on whether rates of parasitism are sufficient to deter native pollinators. Any change in seed set (including increases) of plant species within a community could lead to long-term ecological change, but such effects would be exceedingly hard to detect among the much larger environmental changes that are currently taking place.

21.10 Pollination of Exotic Weeds

As we have seen, both honeybees and bumblebees visit a broad range of flowers. They also appear to prefer to visit exotic flowers (Telleria 1993; Thorp et al. 1994). For example, in Ontario, 75 % of pollen collected by honeybees was from introduced plants (Stimec et al. 1997). In New Zealand, *B. terrestris* has been recorded visiting 400 exotic plant species, but only 19 native species (Macfarlane 1976). The three other introduced *Bombus* species also visit mainly introduced plants (Donovan 1980). In the highlands of New Zealand, honeybees rely almost exclusively on introduced plants for pollen during most of the season (Pearson and Braiden 1990). Introduced *M. rotunda* appear to feed exclusively on introduced plants in Australia (Woodward 1996).

Do visits by exotic bees improve seed set of weeds? In general, rather little is known of the pollination biology of non-native plants, and it is unclear whether inadequate pollination is commonly a limiting factor (Richardson et al. 2000). By virtue of their abundance and foraging preferences, exotic bees often make up a very large proportion of insect visits to weeds. For example, in a site dominated by European weeds in Tasmania, honeybees and bumblebees were the major flower visitors and comprised 98 % of all insect visits to creeping thistle, *Cirsium arvense*. In North America, honeybees increase seed set of the yellow star thistle, *Centaurea solstitialis* (Barthell et al. 2001) and are the main pollinators of two important weeds, purple loosestrife, *Lythrum salicaria* (Mal et al. 1992) and *Raphanus sativus* (Stanton 1987). Donovan (1980) reports that bumblebees are major pollinators of introduced weeds in New Zealand. It thus seems obvious and inevitable that exotic bees will prove to be important pollinators of various weeds (Sugden et al. 1996).

Remarkably, this view has been challenged. It is hard to agree with the conclusions of Butz Huryn and Moller (1995) that “Although honey bees may be important pollinators of some weeds, they probably do not contribute substantially to weed problems”. Butz Huryn (1997) argues that most weeds do not rely on insect pollination, either because they are anemophilous, self-pollinating, apomictic, or primarily reproduce vegetatively. This is undoubtedly true of some weed species.

For example, of the 33 worst environmental weeds in New Zealand (Williams and Timmins 1990), nine fall into one of these categories (Butz Huryn and Moller 1995). However, 16 require pollination and are visited by honeybees, and one is pollinated more or less exclusively by them (the barberry shrub, *Berberis darwinii*). Eight more are listed as having unknown pollination mechanisms (Butz Huryn and Moller 1995). This group includes the tree lupin, *Lupinus arboreus*, and broom, *Cytisus scoparius*, which are self-incompatible and rely on pollination by bumblebees (Stout et al. 2000, 2002). It also includes gorse, *Ulex europeaus*, which is thought to depend on honeybee pollination, and in which seed set is greatly reduced by a lack of pollinators in the Chatham Islands where honeybees and bumblebees are absent (McFarlane et al. 1992). Thus, at least four major weeds in New Zealand are pollinated primarily by exotic bees. *L. arboreus* is currently a minor weed in Tasmania. However, seed set in areas recently colonized by *B. terrestris* has increased dramatically, and it is likely that *L. arboreus* may become as problematic in Tasmania as it is in New Zealand now that it has an effective pollinator (Stout et al. 2002). Its zygomorphic flowers have to be forced apart to expose the stamens and stigma; only a large, powerful bee is able to do this, and no such bees are native to Tasmania. *L. arboreus* is only one of many weeds in Tasmania, New Zealand, and southern Australia that originated in the temperate northern hemisphere and is co-adapted for pollination by bumblebees. Demonstrating that exotic bees increase seed set of weeds is not sufficient in itself to conclusively show that the action of the bees will increase the weed population (Butz Huryn 1997). No long-term studies of weed population dynamics in relation to the presence or absence of exotic bees have been carried out. Because most weed species are short-lived and dependent on high reproductive rates, it seems probable that seed production is a crucial factor in determining their abundance. Key factor analysis of the life history could reveal whether seed set is directly related to population size.

At present, Australia alone has 2,700 exotic weed species, and the control of these weeds and loss of yields due to these weeds cost an estimated AU\$ 3 billion per year (Commonwealth of Australia 1997). The environmental costs are less easy to quantify but are certainly large. Most of these weed species are at present of trivial importance. The recent arrival of the bumblebee may awake some of these “sleepers” weeds, particularly if they are adapted for bumblebee pollination. Positive feedback between abundance of weeds and abundance of bumblebees is probable, as an increase in weed populations will encourage more bumblebees, and vice versa. If even one new major weed occurs in Australia due to the presence of bumblebees, the economic and environmental costs could be substantial.

21.11 Loss of Genetic Diversity: Causes and Consequences

Despite its economic usefulness, biodiversity of Asian hive bee *A. cerana* is suffering precipitous decline and is threatened with extinction in its entire native habitat. For example, in Japan, beekeeping with this native bee species has been completely replaced by European honeybee, *A. mellifera*, and only a few beekeepers and research institutes are raising *A. cerana* colonies (Sakai 1992). In China, out of more than 8.5 million colonies of bees kept in modern hive, 70 % are exotic *A. mellifera* (Zhen-Ming et al. 1992). Similarly, in South Korea, only 16 % beekeeping is with native *A. cerana* and remaining has been replaced by exotic *A. mellifera* (Choi 1984).

In Hindu Kush Himalayan range, beekeeping with *A. cerana* is being replaced by *A. mellifera* at such a fast rate that populations of native *A. cerana* is declining to a level that is no longer viable. These countries include Afghanistan, Bhutan, Myanmar, Nepal, India, Bangladesh, and Pakistan (Verma 1994a). Eva Crane during a visit of some mountain areas of north-west Frontier Province of Pakistan in 1989 concluded that *A. cerana* populations may soon become an endangered species (Crane 1992). Thus, the existing centuries old and long established craft of beekeeping with *A. cerana* has now almost got destroyed in its entire native habitat. *A. cerana* remains till now a forgotten and completely ignored species. Therefore, from biodiversity conservation point of view, it will be disastrous to leave this important genetic resource at its own and research and development interventions is definitely required for its conservation and sustainable uses both in natural and agricultural eco-systems.

21.12 Causes and Consequences of Declining *A. cerana* Diversity

In seeking ways to conserve genetic diversity of *A. cerana*, it is necessary to have a clear understanding of the major threats which this bee species is facing in its own native habitat. Like any other threatened biological resources, decline in *A. cerana* population is also being threatened by human mismanagement, misguided scientific and economic policies, and faulty institutions.

21.13 Major Threat from *A. mellifera*

Many importations of *A. mellifera* in south and south-east Asia have proved disastrous for beekeeping with *A. cerana*. When kept sympatrically, *A. cerana* and *A. mellifera* colonies frequently robbed each other (Koeniger 1982). Another major problem is the transfer of parasites from one species to another. A parasite mite of brood and adults, *V. destructor*, co-exists with *A. cerana* and causes no serious damage to this native bee species. In several countries of Asia, where both these species are kept

together, the parasite has infested *A. mellifera* colonies and has become a serious pest to this unadapted host, which now kills thousands of colonies every year. It is now well documented that through importations of *A. mellifera*, *A. cerana* populations in its native habitat are facing serious risk of extinction.

On the other hand, the native *A. cerana* populations are also threatened by pests and parasites of exotic western honeybee *A. mellifera* for which *A. cerana* is lacking resistance. For example, there are several reports in the literature that Thai Sac Brood Virus disease, European Foul Brood disease, and possibly acarine disease jumped in to *A. cerana* and other Asian bee species from *A. mellifera* in Nepal, India, and other Asian countries killing large number of native bee colonies every year (Rana et al. 2006; Saville 2000; Allen et al. 1990). Pongtap (1990) reported three viral diseases affecting *A. cerana*, namely, Thai Sac Brood Virus, Kashmir Bee Virus, and *Apis* Iridescent Virus, and all these virus diseases probably spread from *A. mellifera*.

Due to these afflictions, populations of *A. cerana* colonies practically reduced to the level of extinction, but through natural selections within two decades, normal population of this bee species stand restored from 10 % of surviving colonies (Reddy 1999; Ge et al. 2000; Ahmad and Partap 2000). These risk factors may vary between different habitat types, landscapes, and bio-geographical region. The relative importance of these factors and in particular their combined effects on *A. cerana* genetic diversity loss are unknown.

Large-scale importations and multiplications of exotic *A. mellifera* into developing countries of south and south-east Asia for better economic returns in terms of higher honey production has also become a myth. This bee species is now so seriously infested with parasitic mites, European Foul Brood, hornets/wasps/birds, and wax moths that beekeeping with this exotic species require intensive treatment with chemicals to control these afflictions, which are very expensive, making this enterprise economically unviable. The intensity and the need for chemical treatment of *A. mellifera* colonies for mites, diseases, and pest control reveal that beekeepers in developing country of south and south-east Asia with large uneducated, ignorant populations in isolated areas are using chemical prescriptions indiscriminately, thus affecting the quality of honey (Verma 2008b).

21.13.1 The Impact of Introduction of *A. mellifera* to Asia

With the development of a beekeeping industry, honeybees, particularly *A. mellifera*, were introduced into many areas of Asia for bee products such as honey, pollen, royal jelly and propolis. However, as the business benefits from the introduction of *A. mellifera* colonies grew, many problems emerged. As mentioned above, these included foraging competition, mating interference, robbing, and the transmission of diseases. The introduction of *A. mellifera* colonies has also had an enormous impact on the native honeybee species in some areas of Asia (Japan: Sakagami 1959; India: Atwal and Sharma 1971; China: Ji et al. 2003; Yu and Han 2003; Yang 2005; Nepal: Partap 1998).

A. mellifera was first introduced in China in the 1920s (Kuang and Kuang 2002). On introduction, this western honeybee proved adaptable to a new environment and not only produced higher yields of bee products but also royal jelly and propolis, which cannot be collected from *A. cerana* colonies because of their extreme low productivity. Since then, this productive species of honeybee began to be widely adopted in Chinese beekeeping.

While enjoying the high profits of these bees, the negative aspects have been widely neglected and few if any had realized the strong impact of *A. mellifera* on the environment and the local honeybees, especially *A. cerana*, until the 1980s. An investigation was launched and conducted by the *A. cerana* Association of China. The results showed that *A. cerana* has become extinct in the Daxing-Anling Forest areas in the northeast and in Xin Jiang Province in the northwest. In the Northeast Plain and North-China Plain areas, all of the *A. cerana* bees in man-made hives have absconded (Yang et al. 1982). In the whole northeast zone, *A. cerana* bees can be found only in the Changbai mountain areas in wild and man-made hives. The plain of drainage area of the Yangtze River where millions of *A. cerana* colonies were kept in the past are now hard to find. In the southern provinces such as Jiangxi, Hunan, Fujian, Guangdong, Guangxi, and Hainan, there are still many *A. cerana* colonies, but their distribution area has shrunk greatly. Compared with those areas above, *A. cerana* colonies in the southwest are in a better condition, particularly in mountainous areas where many *A. cerana* bees can be found living in tree holes, caves, and man-made hives in Yunnan Province and Tibet (Yang et al. 1982).

In conclusion, the introduction of *A. mellifera* caused great losses of *A. cerana* colonies. The population of *A. cerana* colonies is now estimated at not more than one million, a decrease of some 60 % compared with the number before the introduction of *A. mellifera*, and their distribution has shrunk by 75 % (Yang 2005).

In the case of the introduction of *A. mellifera* in Asia, as early as 1959, Sakagami had noticed the impact of *A. mellifera* on *A. cerana* in Japan. In Nepal, Partap (1998) reported that plants and fruits were in shortage of pollination because of the population decrease of *A. cerana* bees, which was caused by the introduction of *A. mellifera*. Even in Europe, with the rapid development of beekeeping at the beginning of twentieth century, many beekeepers preferred to raise some subspecies such as *A. mellifera ligustica* and introduced them from other areas, which caused the local extinction of native subspecies (Ruttner 1988).

Moritz et al. (2005) recognized the severe disaster caused by the introduction of *A. mellifera* to tropical ecological systems and pointed out that local honeybees or other pollinators suffered from the introduced species through food competition or diseases. This resulted in a reduction of biodiversity and an imbalance of the whole ecological system.

During the mating season, both the virgin queens of *A. cerana* and *A. mellifera* can attract heterospecific drones (Yang 2001a; Ji et al. 2003; Wang et al. 2003). However, the *A. mellifera* drones, which are much stronger fliers than *A. cerana* drones, can trap the *A. cerana* queens, although they cannot always mate with them successfully because of the differences in copulatory organs. Their encirclement behaviour can inhibit successful mating between *A. cerana* queens and drones. In

some areas with very many *A. mellifera* colonies, most of the virgin *A. cerana* queens were trapped by *A. mellifera* drones, and only 16 % of *A. cerana* queens were able to mate successfully. More than 80 % of *A. mellifera* queens could successfully mate with conspecific drones, although there was interference by *A. cerana* drones (Wang et al. 2003). This resulted in the population decline of *A. cerana* bees in some areas in recent years. In some areas, they are threatened because their declining population is insufficient to support the community and honeybees are dying out. The decrease or extinction of the native honeybees is a definite threat to the balance of ecology and some plant species could also become extinct because of insufficient pollination (Yang 2005).

21.14 Autochthonous Distribution of *A. mellifera* and its Spread by Humans

The western honeybee was of immense socio-economic value to humans well before the beginning of written history. The oldest records of honey hunting date back to prehistoric cave paintings (Crane 1986), indicating that honeybees have always been an important part of human culture. As long as humans only robbed honey from bees in the wild, natural selection governed the evolution and distribution of the various honeybee subspecies. To date, 26 autochthonous *A. mellifera* subspecies have been identified (Engels 1999; Sheppard and Meixner 2003), and the species covers a biogeographic range that includes western Asia and the European and African continents (Ruttner 1988; Fuchs 1998a, b; Hepburn and Radloff 1998). As for most other organisms, the potential for honeybees to become an invasive species arose in more recent history, when people invented not only containers to house bees (hives) but also tools to easily transport them from one place to another. The technology of long-distance commercial transport across continents and the development of migratory beekeeping over short distances caused the propagation of non-native subspecies of honeybees around the globe under three distinctly different biological conditions:

1. Regions where other subspecies of *A. mellifera* are endemic (Europe, Africa, and western Asia);
2. Regions where *A. mellifera* does not naturally occur, but other species of *Apis* are endemic (central and eastern Asia); and
3. Regions where no other *Apis* species are endemic (America, Australia).

Clearly, in all three cases, invasiveness could have developed. Given the intensity and magnitude of worldwide bee trade, it is actually surprising that very few cases of honeybee invasions that have had negative impact on society and nature have been reported (Goulson 2003; Paini 2004). Much less surprising is that international honeybee trade and transport ensured that pests and parasites of honeybees were also globally distributed (Morse and Flottum 1997). In the following paragraphs, we will not address the spread of diseases, rather discuss invasiveness of *A. mellifera* and its consequences under the three different biological conditions. Goulson (2003)

focused in his review on the scale at which bees have been artificially distributed around the globe. The honeybee, *A. mellifera*, was included but was not discussed in detail. Schneider et al. (2004) recently provided a comprehensive overview on the African honeybee in America, but they did not include invasions elsewhere. We will not review all of the published cases where the impact of introduced honeybees has been studied (this has been reviewed recently by Paini 2004). Instead, we will concentrate on the three best documented cases to illustrate the various modes and scenarios under which honeybees can potentially become invasive.

21.14.1 Regions Where Other Subspecies of *A. mellifera* are Endemic (Europe)

As soon as beekeeping was developed and people provided artificial nesting sites for honeybees, the potential arose for competition between those colonies being kept in near-ideal nest cavities and those colonies that had to survive in naturally shaped cavities. The potential for competition particularly increased when beekeepers developed moveable hives and started transporting large numbers of colonies into proficient honey flows (Crane 1990a, b). Because of the large-scale habitat destruction by agriculture, *A. mellifera* populations in many European countries today are characterized by large human-kept and small wild honeybee populations. Moreover, the anthropogenic introduction of novel pests and parasites (for example, the parasitic mite *V. destructor* (Anderson and Trueman 2000)) further contributed to the decrease of honeybee populations not kept by people (Kraus and Page 1995). Wild colonies compete with the human-kept ones for floral resources, face numerous new diseases introduced by human activities, and are subject to gene flow from human-kept colonies. Because of its specialized mating biology, the honeybee is the only agricultural animal for which the human-kept population has a large potential to share its gene pool with the wild population. Queens mate in flight at drone congregation areas with drones from colonies as far away as 10 km (Evenius 1929), causing a constant gene flow between wild and human-kept populations. Bee breeding was and still is dominated by introducing “superior” honeybees from various parts of Europe and Africa into commercial beekeeping. This practice often overlooks the importance of local adaptation and disregards the need for conserving local subspecies and biodiversity.

As a consequence of this beekeeping practice, native honeybees have been claimed to be extinct and replaced in several parts of Europe (Ruttner 1969; Ruttner 1988). However, recent studies fail to document such complete extinction processes, and autochthonous subspecies can still be identified throughout the European continent (Franck et al. 1998; De la Rua et al. 2001, 2002, 2003). Nevertheless, racial admixture certainly has occurred due to beekeeping activity (Cornuet et al. 1986; Franck et al. 2000; De la Rua et al. 2003). There is no indication, however, that any introduced honeybee subspecies developed invasiveness in Europe. The opposite seems to be true. Massive attempts at completely replacing one subspecies with another

one failed, even when entire beekeeper communities developed strict breeding programs to establish superior breeding lines for apicultural use. For example, German beekeepers tried for many decades to replace the presumably less docile and less productive subspecies *A. mellifera mellifera* with the adjacent *A. mellifera carnica*. Extensive evaluations indicated that carniolan strains were more productive and less aggressive than *A. mellifera mellifera* (Ruttner 1983), which gave rise to one of the most rigorous breeding programs in the history of apiculture. The selection tools were easy, because morphometric analyses allowed for a clear separation between the two subspecies (Ruttner 1983). The cubital index was identified as the prime discriminative tool between *A. mellifera carnica* and *A. mellifera mellifera* (Ruttner 1969). Initially, this morphometric marker reliably discriminated between selected stock and wild honeybees.

However, after several decades of selection, the tool lost efficiency, because non-*carnica* populations maintained by beekeepers also displayed the typical carniolan cubital index, which had obviously introgressed into endemic honeybee populations (Moritz 1991). Clearly, the carniolan race preferred by beekeepers was not invasive, and in spite of hard selection work, endemic honeybee populations can still be identified (see previous text), despite, alas, having lost their low cubital index. Thus, although introgression of imported subspecies did have an impact on “pure” *A. mellifera mellifera* populations, it primarily affected those characters that could be easily selected for. A few populations are maintained by conservation efforts in Europe (for example, *A. mellifera mellifera* on the Danish Island of Laeso; Svendsen et al. 1992; Pedersen 2002). These are worthwhile efforts, but it should be kept in mind that the value of honeybee biodiversity lies primarily in the behavioural and physiological characteristics of the various subspecies and not in a single morphometric data point.

21.14.2 *Africa*

21.14.2.1 Wild and Human-Kept Honeybee Populations

Until recently, the impact of beekeeping on wild honeybee populations in Africa was much weaker than in Europe (Hepburn and Radloff 1998). This is primarily because the proportion of human-kept honeybees is minute in most African countries compared with the wild population. European *A. mellifera* subspecies have been repeatedly imported to the African continent for honey production (e.g., within developmental aid programs). However, colonies of European subspecies did not develop well and disappeared soon after introduction (Onions 1912; Fletcher 1977b; Hepburn and Radloff 1998). Apiculture in Africa is therefore primarily based on endemic wild honeybees. A large number of *A. mellifera* subspecies have been identified (Hepburn and Radloff 1998; Fig. 21.1) throughout the continent. Yet, beekeeping is only poorly developed, queen rearing is virtually absent, and beekeeping mostly relies on using trapped wild honeybee swarms (Swart 2001). Even in the fynbos in South Africa, the region with the highest density of well-developed large-scale beekeeping in Africa

(Johannsmeier 2001), the density of human-kept honeybee colonies is estimated to be well below 1 colony/km² (0.86 km²) (Hepburn et al. 2004). These are outnumbered by far by the number of wild colonies, which has been estimated in the range of 1–4.2 colonies/km² (McNally and Schneider 1996) under various subtropical and tropical conditions. Transport through apicultural activities is, therefore, much less of a problem for African than for European populations, although it is clearly not absent, particularly in the more industrialized regions of the continent.

21.14.2.2 The “Capensis Calamity” in South Africa

Migratory beekeeping in South Africa is well established, and yearly thousands of honeybee hives follow proficient honey flows on trucks of commercial beekeeping operations (Johannsmeier 2001). This posed no problems as long as these migrations were constrained within the endemic ranges of subspecies. However, as soon as endemic honeybees are displaced into the range of other subspecies, the stage for potential invasion is set. Two honeybee subspecies have been identified in South Africa: The Cape honeybee (*A. mellifera capensis*, native to the Cape region) and *A. mellifera scutellata* in the northern regions of the country (Hepburn and Radloff 2002). Glaciations and interglacial periods have produced isolated populations in southern Africa (Deacon and Lancaster 1988). This has been proposed as a mechanism for the separation of honeybee subspecies (Ruttner 1988), which have evolved stable populations providing an excellent example for intraspecific invasiveness. In 1990, the large-scale transport of *A. mellifera capensis* colonies from the Cape Province (Allsopp 1992; Neumann et al. 2002) into the northern provinces by migratory beekeepers had dramatic consequences for *A. mellifera scutellata* beekeeping (Allsopp and Crewe 1993; Neumann and Hepburn 2002). Thousands of *A. mellifera scutellata* colonies disappeared within months because of dwindling colony syndrome (Allsopp and Crewe 1993). It turned out that the dwindling colony syndrome was nothing else but an invasion of parasitic *A. mellifera capensis* workers into *A. mellifera scutellata* populations (Allsopp and Crewe 1993; Hepburn and Allsopp 1994; Neumann and Hepburn 2002). The underlying mechanism was long known (Onions 1912). Workers of *A. mellifera capensis* can produce female offspring through autotaxis (thelytoky; Verma and Ruttner 1983; Crozier and Pamilo 1996; Lattorff et al. 2005) and can establish themselves as pseudoqueens that produce queen-like pheromones and have activated ovaries (Hemmling et al. 1979; Crewe and Velthuis 1980; Ruttner and Hesse 1981; Velthuis et al. 1990). These pseudoqueens can switch to a socially parasitic life cycle by entering a foreign colony and producing parasitic worker offspring through thelytokous parthenogenesis (Neumann and Hepburn 2002). *A. mellifera scutellata* queens can actively search for these social parasites and engage in a physical lethal fight using their stingers (Moritz et al. 2003). Usually, the queen wins these fights, but parasitic workers sometimes kill the queen. When many social parasites are in a colony, the host queen will eventually lose a fight and the winning *A. mellifera capensis* worker will take over the colony. The host colony will now exclusively raise parasitic worker offspring of the *A. mellifera capensis*

social parasite, which eventually causes the collapse of the colony (Neumann and Hepburn 2002; Swart 2003). If the majority of *A. mellifera scutellata* workers are replaced by parasitic workers, regular brood rearing and foraging ceases (Martin et al. 2002) because most *A. mellifera capensis* workers invest in individual reproduction (Hillesheim et al. 1989). As a result, eggs are laid but none is reared to the adult stage. In contrast to usurpations by queenright *A. mellifera scutellata* swarms in the Americas (Schneider et al. 2004), takeover by worker social parasites results in the death of the host colony and in a complete loss for pollination purposes.

The Capensis calamity presents an extraordinary example of an invasion because all parasitic workers, which can be found in vast areas in South Africa, are offspring of a single laying worker (Baudry et al. 2004). The mechanism is believed to be well understood: laying workers produce clonal offspring with reduced recombination (Moritz and Haberl 1994; Baudry et al. 2004). This is due to a central fusion of the meiotic products during automixis (Verma and Ruttner 1983) as well as an extreme reduction of crossing over (Baudry et al. 2004).

When more than one parasitic worker is in a colony, there is fierce competition to become the reproductive dominant one (Moritz et al. 1996). Intracolony selection will favour the most adapted parasitic worker, i.e. the worker that most quickly develops a queen-like pheromone signal will suppress others (Moritz et al. 2004). As a consequence, only the most adapted genotype will prevail. Because the offsprings of this worker are clonal, they will have the identical genotype. Highly specific co-adapted gene complexes will not get distorted, because modifications through recombination or mutation events are rare or lacking (Baudry et al. 2004). This most adapted genotype will thus stay intact as long as it finds new host colonies (Neumann and Moritz 2002). Indeed, the parasitic *A. mellifera capensis* worker population shows the genetic signature of a clone in vast regions of northern South Africa (Baudry et al. 2004).

While the beekeeping industry was heavily affected (colony losses of up to 100 % each year; Greeff 1997; Swart 2003), the invasion of parasitic *A. mellifera capensis* workers into the wild *A. mellifera scutellata* population appears to be much less of a problem. Population ecological modelling showed that unless colony densities in the wild were high, it would be very unlikely that parasitic workers would spread into the wild population (Moritz 2002). The transmission potential of parasitic workers is rather low and they preferably infest colonies in close proximity. This is typical for apiaries but not for wild colonies. In addition, legislative regulations have to date prevented the massive spread of *A. mellifera capensis* parasites into wild *A. mellifera scutellata* populations.

Under natural conditions, high virulence often is at the expense of a reduced transmission rate. The underlying evolutionary mechanism has been termed short-sighted selection (Levin and Bull 1994). As a result, the highly virulent parasite worker should have a reduced transmission rate, reducing its fitness. Although this could also be observed in *A. mellifera capensis*, transmission is not a problem for the virulent clone because the beekeeper's operations facilitate the spread of the parasite from colony to colony. Indeed, the invasive spread of *A. mellifera capensis* social parasites is promoted by migratory beekeeping practice (Johannsmeier 1983;

Neumann and Moritz 2002). This is similar to the jump dispersal of the invasive Argentine ant (*Linepithema humile*), which was also mediated by humans (Suarez et al. 2001). Both the susceptibility of the *A. mellifera scutellata* host (Neumann and Hepburn 2002) and the human-mediated spread of the socially parasitic workers seem to contribute to the invasiveness of *A. mellifera capensis* within the range of the neighbouring subspecies.

In conclusion, socially parasitic workers of *A. mellifera capensis* are destructive to human interests by destroying a high proportion of the human-kept *A. mellifera scutellata* population each year. Because the vast majority of *A. mellifera scutellata* colonies are wild and the impact on such host populations is minor so far (Moritz 2002), the effects on biodiversity are probably small. However, the potential of *A. mellifera capensis* to become invasive, with larger effects on biodiversity, should not be ignored. Indeed, at least four other honeybee subspecies are susceptible to infestations by socially parasitic workers (*A. mellifera ligustica*, *A. mellifera carnica*, *A. mellifera caucasica*, *A. mellifera mellifera*; Onions 1912; Koeniger and Würkner 1992; Woyke 1995). This may result in significant damage to biodiversity in regions where the majority of honeybee colonies are human-kept (e.g., in Europe).

21.14.3 Regions Where *A. mellifera* Does not Naturally Occur, but Other Species of *Apis* are Endemic

Introductions of European *A. mellifera* into Asia for honey production have had mixed success. Recently, beekeeping in China has been concentrating on *A. mellifera mellifera*, causing a considerable decline in *A. cerana* beekeeping (Li 1998). The problem could potentially be grave, because drones and queens of both species readily mate but do not produce viable offspring (Ruttner and Maul 1983). *A. cerana* queens may thus have poor mating success if many *A. mellifera* drones are present. Indeed, Li (1998) showed that *A. cerana* mating success was influenced by *A. mellifera* drones, sometimes causing a complete lack of successful matings of *A. cerana* queens. Competition for floral resources with other bees in general and nest site competition between *A. mellifera* and the other cavity-dwelling *Apis* species in Asia (*A. cerana*, *A. koschevnikovi*, *A. nigrocincta*, and *A. nuluensis*) in particular may also occur (for a recent review on the relatively few documented cases of the interactions between introduced *A. mellifera* and wild bees in Asia, see Paini 2004). Whether this competition results in true invasions sensu stricto, Lonsdale (2004) may be questioned because introduced *A. mellifera* also suffer from competition with native species. In regions where other honeybee species naturally occur, *A. mellifera* colonies are often out-competed. For example, the native honeybee species *A. cerana* and *Apis dorsata* negatively affected the growth of European *A. mellifera* colonies in a forest ecosystem through aggression and robbing of stores (Manila-Fajardo and Cleofas 2003). In spite of the diversity in the forest ecosystem, *A. mellifera* failed to exploit the nectar and pollen sources of most plant species (Manila-Fajardo and Cleofas 2003). Moreover, competition for nectar between *A. mellifera* and *A. cerana*

has been shown in feeding experiments (Sakagami 1959). Introduced European *A. mellifera* often suffer from novel parasites and diseases. For example, the devastating global spread of the parasitic mite *V. destructor* (Anderson and Truman 2000; Oldroyd 1999) resulted from *A. mellifera* introductions into Asia and a host shift from *A. cerana* to the western honeybee. In this light, it may not be surprising that there are no reports yet of large feral populations of *A. mellifera* in Asia despite repeated introductions. This suggests that the introduction of European subspecies of *A. mellifera* have not caused invasions with large effects on biodiversity in Asia so far. But this does not exclude the possibility that introductions of African subspecies, which are better adapted to tropical conditions, may result in quite a different scenario (see later).

21.14.4 Regions Where No Other *Apis* Species are Endemic: Intraspecific Competition with Non-Indigenous Pollinators the “African” Honeybee in America

The early European settlers introduced *A. mellifera* to the Americas about 250 years ago for honey production (Nogueira-Neto 1972; Sheppard 1989). Since honeybees are not fully domesticated animals, swarms escaped into the wild and established a solid and sustainable feral honeybee population in the temperate regions of the American continent (Spivak et al. 1991). This feral population has been termed “mixed European origin” or “New World population” because several European subspecies (e.g., *A. mellifera carnica*, *A. mellifera ligustica*, *A. mellifera mellifera* among others) were introduced to a varying extent. However, in the tropics of South America, introduced European honeybees were far less successful than in North and Central America. The colonies imported from Europe were often only poorly adapted to tropical conditions. Colonies did not gain desired productivity levels (Delgado and del Amo 1984), giving rise to the plausible plan to import tropical African rather than European honeybee subspecies for honey production. This plan caused the most dramatic example of honeybee “invasiveness”: the spread of African honeybees in the Americas in the second half of the last century.

21.15 Exotic Pollinators

The accidental or deliberate importation of pollinators into new geographic regions has lately become a concern to some environmentalists. The widespread European honeybees and bumblebees imported into some regions (Dunning 1886) may be displacing native bees or other pollinators of the native flora, or they may provide unsatisfactory pollination of these plants (Pyke and Balzer 1983; Vogel and Westerkamp 1991; Westerkamp 1991; Wilson and Thomson 1991; Thomson and Thomson

1992; Kato 1993; Paton 1993). *B. terrestris* (L.) is now widely distributed for pollination of crops and is another potential competitor (Donovan 1990; Semmens et al. 1993; Kato 1993). Although several species of solitary bees have been distributed to new regions for pollinating crops, they are less likely to displace native bees than is the ubiquitous European honeybee, because of their greater host specificity, climatic limitations, shorter foraging ranges, specific conditions for nesting, and brief adult life (Donovan 1990). The principles that apply to the importation and establishment of exotic pollinators resemble those of classical biological control (Batra 1982), in which beneficial organisms are sought near the centre of origin of the problem-causing organism in a similar climate, observed and tested for lack of harm to other beneficial or rare organisms, imported into quarantine where their parasites can be eliminated, tested with hosts again, in confinement and then released for a specific purpose into their new environments. Considerable effort has gone into identifying the most efficient pollinators of several Eurasian crops that may be worthy of importation into North America, South America, Australia, and New Zealand where native pollinators are not well adapted to pollinate these crops (Parker et al. 1987). Conversely, American pollinators of sunflowers, cotton, passion fruit, squashes and gourds, avocado, tomato, chilli, blueberries, and cranberries have been studied in areas where these crops are believed to have originated, the goal being to introduce their pollinating bees into areas where the crops are now grown without their most efficient pollinators (Parker et al. 1987).

Large human populations can currently only be maintained by agriculture, which must alter native environments. Humans, like all organisms, may be expected to increase in population (absent natural enemies), until all available resources are fully exploited, either directly (as in agriculture) or indirectly, for example, when biota and other natural resources have been assessed and then reserved for possible future value (in economic terms, internalized). The impact of humans is large, complex, and irreversible (Vitousek 1994). One way to provide for more habitat to conserve native bees and other biodiversity, as well as non-renewable resources, would be to improve the yields on existing crop lands. This can be accomplished, in part, by introducing the most efficient pollinators for these crops.

The large bumblebee, *Bombus* (L.), is naturally distributed in Europe and adjacent territories, including England, most of Scotland, the north coast of Africa, southern Scandinavia, major Mediterranean Islands, and some Atlantic Islands (Madeira and the Canary Islands) (Estoup et al. 1996; Chittka et al. 2004; Velthuis and van Doorn 2006). Since *B. terrestris* became commercially available as a valuable pollinator of greenhouse crops in the late 1980s, this species has been shipped throughout the world in vast numbers (Goka et al. 2001; Hingston et al. 2002). The invasive potential of *B. terrestris* has been suggested by its successful naturalization in New Zealand (Donovan and Wier 1978; Donovan 1980; MacFarlane and Gurr 1995; Goulson and Hanley 2004), Israel (Dafni and Shmida 1996; Dafni 1998), and Chile (Ruz 2002). In Japan, *B. terrestris* has been used for pollination of greenhouse crops, particularly tomatoes (*Solanum lycopersicum* L.), since 1991 (Ono 1998). Importation of *B. terrestris* colonies has been increasing annually, and approximately 70,000 colonies were used in 2004 (Kunitake and Goka 2006). Since the introduction

of *B. terrestris* to Japan in 1991, researchers have warned about the strong potential impacts on native bumblebee species and mutualistic pollination systems (Kato 1993; Washitani and Morimoto 1993; Washitani et al. 1997). Unfortunately, the warnings are becoming real. In 1996, a naturally occurring nest, which was the first evidence of naturalization of *B. terrestris* in Japan, was found in Monbetsu in the Hidaka region of Hokkaido, northern Japan, close to the agricultural area where *B. terrestris* was mass introduced (Washitani 1998). Since then, increasing numbers of *B. terrestris* queens emerging from hibernation have been recorded around the area, and this species had become dominant in the bumblebee fauna by 2003 (Matsumura et al. 2004). Monitoring in the Hidaka region revealed that *B. terrestris* foraged on the flowers of approximately 100 species, of which 40 were native, suggesting potential competition for floral resource with native bumblebees (Matsumura et al. 2004). Mass infestation of *B. terrestris* queens was observed in 2003 in the Iburi region of Hokkaido, about 10 km away from a large source of bumblebees introduced for greenhouse tomato cultivation (Yokoyama et al. 2004). The *B. terrestris* observed in the Iburi region appear to be naturalized, and this species could have adverse effects on native flower-visiting insects through competition, either for floral resources (nectar and pollen) or for nest sites. Inoue et al. (2007) reported that there were considerable niche overlaps in flower resource use between introduced *B. terrestris* and native *B. hypocrita sapporoensis*/*B. pseudobaicalensis*. They suggested that competition for nest sites rather than flower resources is the major ecological mechanism for displacement of native bees. The large reduction of *B. hypocrita sapporoensis* queen indicates that *B. terrestris* may cause local extinction of native bumblebees. Control of established *B. terrestris* populations and prevention of further range expansion are urgently needed.

21.16 Impact of Importations on Native Bees

There have always been efforts to acquire races of bees which could serve as good honey producers, pollinators with better qualitative and quantitative characters. This has necessitated importing good bee stocks from one country to another. There are instances where these imports made by observing proper quarantine measures had contributed substantially to the economy but unwanted races of honeybees have caused serious concern for beekeeping and public health. Importation of honeybees and other pollinators that are not indigenous to any country may endanger local honeybees and other pollinators. The inherent problems of accidental entry of parasites, pests and diseases and undesirable *Apis* spp. to non-existent areas, thereby threatening the existence of honeybees and other local pollinators, have been reviewed by Gatoria et al. (2000) and Abrol (2001). The competition between existing honeybees and exotic species may lead to the elimination of indigenous species, e.g., introduction of *A. mellifera* eliminated *A. cerana japonica* in Japan and the fate of *A. cerana indica* in India is not different. Therefore, there is a need of legislative measures to control the entry of pests and diseases (Table 21.2).

21.17 Import of Pollinators

Import of *M. rotundata* F. in Bulgaria from France and Canada proved to be effective pollinator of Lucerne (Dochkova 1984). Similarly, Aballay et al. (1986) reported the successful establishment of *B. ruderatus* (F.) imported from New Zealand into Chile for pollination of red clover (*Trifolium pratense* L.). Following the success of some earlier introduction of bumble bees for pollination of red clover in New Zealand, two bees *M. rotundata* and *Nomia melanderi* Cockerell were introduced as pollinators of Lucerne (Donovan 1990). Richards and Kronic (1990) imported *M. rotundata* bees from Canada for pollination of alfalfa (*Medicago sativa* L.) in Yugoslavia which also proved worthwhile. Keven and Laverty (1990) cautioned in Canada that parasitic mite like *Varroa jacobsoni* Oudemans can survive on imported pollinators or on non-honeybee insects that may come in contact with infested honeybees visiting these flowers in their native country. Hence, the import of pollinators is made through strict quarantine procedures after ensuring them free from such parasites.

21.18 Import of Honeybee Packages and Queen Bees and Spread of Enemies and Diseases

In order to reduce the risk of introduction of *V. jacobsoni*, the import of honeybees from USA into Canada was prohibited in 1987 (Switzer 1993). During 1988, Canada closed the border against import of queen bees from USA to prevent and slow down the introduction of *Acarapis woodi* Rennie and *V. jacobsoni*. The act was widely supported by Canadian beekeepers as it resulted in the annual saving of \$ 3 million on account of miticidal treatment (Winston 1994). During 1992, *V. jacobsoni* was detected in feral colonies of Asian honeybees (*A. cerana* F.) on Dauan island adjacent to Papua New Guinea (Australian territory). The island was immediately declared as infested zone and adjacent areas as surveillance zones, monitored by Australian quarantine and inspection service (AQUIS). Schrader and Reid (1986) stated that it was because of strict quarantine rules that ensured the absence of American foulbrood, Braula, and parasitic mites in New Zealand.

Deodikar (1971) attributed the first reported acarine disease (*Acarapis woodi*) in 1950's in India to the importation of *A. mellifera* and suggested that quarantine law should be made applicable to bees also. Kshirsagar et al. (1981) suggested the need of setting up of a quarantine procedure on offshore island in order to prevent the entry of honeybee diseases and enemies into the country. Routine monitoring in Georgia (USA) detected *A. woodi* in April 1985. The infested apiary was destroyed and quarantine of all apiaries within 3.2 km imposed (Hall et al. 1987). There were no reports of occurrence of *A. woodi* in Finland until 1991. During the summer of 1991, many queen bees were imported from USA with proper health certificates. When attendant workers of nine queens from a batch of 300 queens were examined, 17 out of 52 bees were found to be infested with *A. woodi* (Korpela and Fakhimzadeh 1991), thus putting a question mark even on the validity of health certificates. Whitten et al. (1979) discussed the establishment of rigorous quarantine procedures in Australia to minimize the risk of the introduction of exotic diseases and parasitic mites.

About one quarter of a million mated queens were shipped to western Canada each year from USA (Jay and Dixon 1982). However, 0.5–18 % of imported queens were infected with *Nosema apis* Zanders. Later on, reports of occurrence of *Nosema apis* and amoeba (*Malphihamoeba mellificae* Prell) were reported by Liu et al. (1987). Liu et al. (1991) reported the isolation of three Australian strains of Kashmir bee virus from bees in different parts of the Australia and cautioned the import of bees as the infected bees carry the virus in inactive phase for long.

Bacterial diseases like American foulbrood (Singh 1961) in Uttar Pradesh, India, and European foulbrood in Maharashtra (Diwan et al. 1971) from *A. cerana* have been reported, but thereafter, neither *A. cerana* nor in *A. mellifera* bacterial diseases were found till 2000. Recently, European foulbrood has been detected in Himachal Pradesh, Punjab, and Jammu and Kashmir from *A. mellifera* (Anonymous 1999, 2000a, b; Abrol, 1996) and from *A. cerana* in Jammu and Kashmir (Abrol and Smith, unpublished data) which could be consequence of illegal trafficking of the queen bees into India from across the border, thereby necessitating strict quarantine in India. Brown (1979) has reported the occurrence of European wasp *Vespula germanica* L. nests in Sydney and New South Wales during 1978. The wasps were seen in large numbers in the vicinity of apiaries.

21.19 Introduction of unwanted races of honeybees and their impact on native bees

21.19.1 Africanized/Killer Bees

Accidental release of Africanized honeybees (AHB), *A. mellifera scutellata* (*A. mellifera adansonii* Laterille) in Brazil during 1957 is one of the most debated problem. Because of its defensiveness and aggressive temperament, it has created a serious public nuisance and has been designated as a killer bee. Stibick (1987) advocated very hard steps ranging from destruction of swarms to aerial spraying of insecticides. The AHB has spread throughout the south and central America and, at present, is heading northwards from southern USA, replacing European honeybee races in many countries (Cobey 1999).

21.19.2 Cape Bee Problem

Allsopp and Crewe (1955) reported the invasion of AHB (*A. mellifera scutellata*) colonies by the cape bees (*A. mellifera capensis* Escholtz) and their usurpation of *scutellata* queens. This phenomenon was later on dubbed as “cape bee problem”. The invasion of AHB colonies by cape bees resulted in dwindling and dying of 50,000–100,000 bee colonies since 1991 from Transvaal and north cape region of Africa (Allsopp 1993). During 1993, legislation was set up under agricultural pest act 1983 (Act No. 36) to prevent movement of cape bees into zone of AHB (Transvaal region). All cape bee colonies already in that region were eradicated under this act (Keetch 1993).

Roubik (2009) found that the exotic Africanized honeybees did not produce a negative effect on native bees, including species that were solitary or highly eusocial. Major differences over time were found in honeybee abundance on flowers near habitat experiencing the greatest degree of disturbance, compared with deep forest areas. At the population level, there was no sudden decline in bees after AHB arrival and relatively steady or sinusoidal population dynamics. However, the native bees shifted their foraging time or floral species. A principal conclusion is that such competition is silent, in floristically rich habitats, because bees compensate behaviourally for competition. Other factors limit their populations. Leigh et al. (2009) reported that colonies, like human civilizations, respond to increased competition with both increased specialization and generalization. It was interesting to find that Africanized honeybees seldom or never skirmish with or show any aggression towards other foragers on flowers. (Roubik 1978, 1980, 1982a, b, 1983, 1988, 1989, 1990, 1991, 1996a). The European honeybees, compared with Africanized honeybee colonies in the same apiaries, displayed a quantitative overlap in pollen species harvest of only 55 %. They also are best regarded as distinct races of *A. mellifera* (Francoy et al. 2008). More important, the European honeybees chose to specialize, i.e. focus a major effort in forage collection, on considerably fewer species than the AHB. The European bees under direct competition for food with AHB were 33 % more specialized, and their pollen use included 33 % more species. Evidently, the European bees were both more generalized and specialized than before the pressure of competition from a clearly more dominant (and abundant) honeybee was upon them. The total estimated pollen species used by *A. mellifera* included about 20 % of the 860 local vascular plant flora. Curiously, one species of nectarless tree (*Cecropia peltata*) was the dominant pollen type used by both races of *A. mellifera* during experiments, whereas it was barely present in the pre-AHB period.

21.20 Conclusions

Both *A. mellifera* and *B. terrestris* are now abundant over large areas where they naturally did not occur. They are both polylectic and thus use resources utilized by a broad range of native species. Various Megachilidae have been introduced to North America and one species to Australia and New Zealand, but very little is known about their impacts.

It seems almost certain that abundant and widespread exotic organisms that single-handedly utilize a large proportion of the available floral resources do impact on local flower-visiting fauna. Consider, for example, the Tasmania native bee community. One hundred and eighty years ago, this presumably consisted of a large number of small, solitary, and subsocial species. Over 100 species are known to be present today, and many more probably exist. Nowadays, by far the most abundant flower-visiting insects at almost every site is the honeybee, often outnumbering all other flower-visiting insects by a factor of 10 or more (D. Goulson, unpublished data). In the southeast, the second most abundant flower visitor is usually the bumblebee, *B. terrestris*. The majority of floral resources are gathered by these bees, often during

the morning before native bees have become active. It is hard to conceive how the introduction of these exotic species and their associated pathogens could not have substantially altered the diversity and abundance of native bees. Unfortunately, we will never know what the abundance and diversity of the Tasmanian bee fauna were like before the introduction of the honeybee. Of course the same applies to most other regions such as North America, where the honeybee has now been established for nearly 400 years. It is quite possible that some, perhaps many, native bee species were driven to extinction by the introduction of this numerically dominant species or by exotic pathogens that arrived with it. Even if it were practical or considered desirable to eradicate honeybees from certain areas, it would be too late for such species.

Similarly, the introduction of exotic bees must increase seed set and hence weediness of some exotic plants, particularly when, as in the case of the bumblebee in Australia, many of the weeds were introduced from the same geographic region and are co-adapted with the introduced bee. It must be remembered that introduced bees provide substantial benefits to man in terms of pollination of crops, and in the case of the honeybee, in providing honey. These quantifiable benefits should be weighed against the likely costs. In areas where weeds pollinated by exotic bees are a serious threat, and/or where native communities of flora and fauna are particularly valued, it may be that the benefits provided by these species are outweighed by the costs. Clearly further research, particularly rigorous manipulative experiments, are needed to determine how much introduced bees contribute to weed problems and whether they do substantially impact upon native pollinator communities. The cautionary principle argues that in the meantime we should at the very least prevent further deliberate release of exotic bee species (such as of bumblebees in mainland Australia, and speculative introductions of various solitary bee species in the USA). Unlike many of the other impacts that man has on the environment, introduction of exotic species is usually irreversible. It would also seem sensible to avoid placing honeybee hives within environmentally sensitive areas where possible, particularly areas where the native flora is threatened by invasion with weed species.

Despite the paucity of research investigating the question of honeybee/native bee competition, researchers have started to realize the importance of assessing direct measurements. Indirect measurements can assess rapidly the potential for competition, but direct measurements are needed to confirm competition. A more focused approach using experiments with increased replication and over several seasons (Sugden et al. 1996) could give the answers that are currently required.

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Chapter 22

Conservation Strategies

22.1 Introduction

In the 100 years between 1880 and 1980, the South and Southeast Asian nations of India, Bangladesh, Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, Singapore, Brunei, Indonesia, and the Philippines, grew in human population by 262 %, the area of cultivated land by 86 %, the area bearing grass and shrub vegetation by 20 %, while total forest cover decreased by 29 % (Flint 1994). Deforestation has continued unabated during the last 25 years (Sodhi et al. 2004). The region has developed an extremely high human population density, and in some countries such as Pakistan, Nepal, and Bangladesh, rapid population growth continues today (The Department of Economic and Social Affairs of the United Nations 2004). Increasing human population size, especially when coupled with increased affluence and per capita consumption inevitably causes increased pressures on natural ecosystems. Of particular concern for honey bee conservation is broad scale conversion of primary forest to short-cycle forestry, rubber and oil palm plantation, agriculture, and urban areas (Kevan and Viana 2003; Sodhi et al. 2004). All these activities involve removal of mature trees suitable for nesting, and often involve reduction in food resources and the use of pesticides. In some cases, direct interactions with humans can result in nest destruction (Underwood 1992). Increasing population and affluence coupled with a desire for natural products harvested from the wild can also increase economic incentive for hunting and gathering within the remaining forests (Nath et al. 1994; Chen et al. 1998; Wilkie and Carpenter 1999; Nath and Sharma 2007). Despite the foregoing discussion, indigenous honey bees remain common throughout much of their original range. The red dwarf honey bee *Apis florea* is actually expanding its range into the Middle East (Mossagegh 1993) and the Eastern hive bee *A. cerana* into New Guinea (Anderson 1994). In Hong Kong, one of the most urbanized and altered landscapes on the planet, *A. cerana* remains common, and is an important pollinator of remnant vegetation (Corlett 2001). Nonetheless, there are obvious signs of threatening processes at work on some species in some areas, and it is suspected that these processes either have driven or soon will drive local extinctions. Perhaps this has already occurred in the dwarf bees on the island of Hong Kong where they are apparently absent (Corlett 2001). The red honey bee

A. koschevnikovi is now extremely rare on peninsular Malaysia and the south of Thailand (Otis 1996). It is not clear whether complete extinction of a particular species is likely or possible, but the threat is real and potential consequences of such an extinction are significant.

Oldroyd and Nanork (2009) reported that East Asia is home to at least nine indigenous species of honey bee. These bees are extremely valuable because they are key pollinators of about one third of crop species, provide significant income to some of the world's poorest people, and are prey items for some endemic vertebrates. Furthermore, Southeast Asian Dipterocarp forests appear to be adapted to pollination by honey bees. Thus, long-term decline in honey bee populations may lead to significant changes in the pollinator ecology of these forests, exacerbating the more direct effects of deforestation and wood harvesting on forest health. Although complete extinction of any honey bee species is seen as unlikely, local extinction is likely to occur across extensive areas. The most significant threats to local honey bee populations are deforestation and excessive hunting pressure. Conservation of East Asian honey bees requires immediate action to determine what rate of colony harvesting by honey hunters is sustainable. This requires information on the demography of hunted populations, particularly the intrinsic growth rates and the rates of harvest.

Himalayan region is rich in honeybee species and genetic diversity. Amongst the different native honeybee species, *Apis cerana* is equivalent to European honeybee *Apis mellifera* because both can be domesticated and can build parallel combs. Our research group in International Centre for Integrated Mountain Development (ICIMOD) has successfully identified genetic variance in morphological characters of *A. cerana* and these results reveal the occurrence of three subspecies in the Himalayan region named as *Apis cerana cerana*, *Apis cerana himalayensis* and *Apis cerana indica*. Each subspecies has further locally adapted geographic ecotypes which differ from each other in several biological and economic characters. Despite its economic usefulness, beekeeping with *A. cerana* is still underdeveloped throughout its range. The major threat comes from its replacement with exotic and more prolific *A. mellifera*, habitat alteration, pesticide poisoning, diseases, and enemies particularly the recurrence of sacbrood virus disease and human predations especially through honey hunting methods. Such decline is undesirable in terms of its commercial value, maintenance of biodiversity in natural ecosystems, and productivity of farming systems. In order to have full utilization of this natural resource, there is a need for adoption of conservation strategies.

22.2 Value of Honey Bees

Although we would argue that honey bees have intrinsic value, it is often useful in the conservation context to be armed with the tangible benefits of a species so that they may be given higher priority for conservation policy and perhaps funding (Chardonnet et al. 2002).

22.2.1 Pollination Services

Although most of the heavily traded agricultural commodities derive from plants that are self pollinated, wind pollinated, or propagated vegetatively, upto a third of the food we eat is derived from plants which are either dependent on or benefitted from insect pollination, especially by honey bees (e.g., Williams 1996; Richards 2001; Klein et al. 2007). The value of crops pollinated by the western honey bee *A. mellifera* is staggeringly large (e.g., Scott-Dupree et al. 1995; Morse and Calderone 2000; Gordon and Davis 2003), but unfortunately, no estimates are available for the value of honey bee pollination for Asian counties or for Asia in aggregate. Natural ecosystems are also heavily dependent on animals for pollination (Bawa 1990; Corlett 2004). There is increasing concern that anthropogenic disruption of plant pollinator mutualisms will lead to a wave of plant extinctions (Bond 1994; Buchmann and Nabhan 1996; Biesmeijer et al. 2006). Because of their dance language and large foraging range, honey bee colonies can rapidly identify and exploit ephemeral floral resources over a wide area (Koeniger et al. 1982; Dyer 1985; Punchihewa et al. 1985; Dyer and Seeley 1991; Dornhaus and Chittka 1999; Sen Sarma et al. 2004; Dornhaus et al. 2006; Beekman and Lew 2007; Beekman et al. 2008), often resulting in inter-specific competition for food (Koeniger and Vorwohl 1979; Oldroyd et al. 1992; Rinderer et al. 1996; Köppler et al. 2007). Perhaps for this reason, the non-*Apis* bee fauna of Asia is depauperate relative to tropical forests in Australia and America (Michener 1979; Corlett 2004; Batley and Hogendoorn 2009). The forest communities of tropical Asia evolved with two or more honey bee species present, and may therefore be particularly vulnerable to a reduction in the density of honey bees (Corlett 2004).

Crop pollination is an essential ecosystem service, which is efficiently provided by different pollinators. It is commonly believed that nearly 70 % of cultivated crops all over the world are cross-pollinated and depend on insects like honey bees for pollination. Dwindling population of such useful pollinating insects has now become a global problem. The importance of bees is often underlined with their role in pollination services and income generation by production of honey and there has been a common concern that population of indigenous bees are declining at an alarming rate. Amongst various pollinators available, Dyer and Seeley (1991) reported that *A. cerana* shows a disproportionately high mass-specific metabolic rate, their foragers make many more trips per day in the same habitat than do foragers of the other species. *A. cerana* can therefore be considered as one of most efficient pollinators. *A. cerana* is widespread in temperate and tropical Asia. There are many different subspecies and races of *A. cerana*, due to the wide range of habitats from temperate mountain regions to tropical islands it occupies. *A. cerana* is a vital component of natural ecosystem. Its decline may have serious consequences for various entomophilous plant species. This bee species shows distinct advantages over *A. mellifera* for pollination of agricultural crops. These include longer foraging hours, earlier initiation of foraging activity even at 5 °C outside environmental temperature, short flight range, low cost of colony management during earth periods, no foraging competition with other native bee

species, non-*Apis* pollinators and coevolution of this bee species with native crops etc. (Verma 1992). Keeping this in view, promotion of beekeeping with *A. cerana* with a domestic setting appears to be essential for the maintenance of biodiversity of forest and grassland ecosystem and for enhancing the productivity of farming systems. Unfortunately, in developing countries of South and Southeast Asia, the role of bees and beekeeping as an important biological input for enhancing the yield of agricultural crops has often been underestimated. The sustainable development of agriculture in the twenty-first century will therefore necessitate reorientation of the present crop production technologies. Instead of making substantial use of physical inputs such as chemical fertilizers, biocides, irrigation, heavy machinery, etc., a shift towards biological inputs such as bee cross-pollination, biological nitrogen fixation nutrient uptake, biotechnology etc. will become essential to increase food productivity. Moreover, such biological based agriculture will have only positive ecological consequences. Thus, there is a need to create awareness amongst policy makers, planners, and aid agencies about promoting bees and beekeeping as an important component of present-day strategies for sustainable agriculture and rural development program (Verma 1990).

22.3 Honey Bees as Prey

Asian honey bees are prey for a variety of insect, mammalian, and bird predators (Oldroyd and Wongsiri 2006). Several bird species are specialist predators of honey bees including the Orange-rumped honey guide (*Indicator xanthonotus*), the Malaysian honey guide (*I. archipelagicus*) and the Oriental (*Pernis ptilorhyncus*) and Barred (*P. celebensis*) honey buzzards. Still others, particularly the bee-eaters (*Merops* spp.) and drongos (*Dicrurus*), are opportunistic hunters of Asian honey bees. These species would either be imperilled or driven to extinction if Asian honey bees were themselves made extinct.

22.4 Social and Religious Values

Many Asian people revere honey bees, and are concerned for their welfare. Honey bees play an important role in two of the main religions of Asia. In the Hindu religion, honey represents the ‘blendedness of everything’ and is often mixed with clarified butter, sugar, milk, and curd to produce one blended mixture, which is shared amongst participants in ceremonies. Although not as central, honey bees feature in a variety of religious stories from Buddhism as well. Stories about bees are used to teach people the value of working hard, flying low (being modest), being clean, being clever in collecting, and being united as a family.

22.5 The Values of Conserving *A. cerana* Biodiversity

The natural products which honeybees produce and humans now use are honey, royal jelly, pollen, propolis, beeswax, and bee venom. These materials have been widely used as nutritional food and for medicinal and pharmacological purposes since ancient times. These natural products of beekeeping industry have both consumptive and productive use value because these can be consumed effectively without passing them through a market and at the same time these hive products can be commercially harvested for exchange in formal markets throughout the world. The common man is well aware of the direct values of this important biological resource.

However, besides these direct values, honeybees provide non-consumptive benefits of conserving botanical resources which may far outweigh direct values when they are computed. These social insects deal with primary functions of ecosystem such as reproduction, including pollination, gene flow, cross-fertilization, maintenance of biodiversity, environmental forces and species that influence the acquisition of useful genetic traits in economic species and maintenance of evolutionary processes, leading to constant dynamic tension amongst the competitors in ecosystems. These non-consumptive benefits can be harnessed to maximum extent through the use of native bee species like *A. cerana* than with exotic *A. mellifera*.

Due to displacement of indigenous, well adapted subspecies of *A. cerana*, we will soon lose these genetic resources. This objective is fully in accordance with the requirements of International Convention on Biological Diversity which specifically cites pollination as a key ecosystem function that is threatened globally. Loss of genetic diversity of *A. cerana* in its native habitat in Asia has very serious economic and ecological consequences because it will not only add fragility to the existing ecosystem but millions of vulnerable farmers in this region will lose their sustainable livelihood. We, therefore, need a concept for preserving honeybee subspecies and their ecotypes as genetic resources for future demand. The preservation of biodiversity and the enhancements towards adaptation in the honeybee should not be mistaken for a mere altruistic chore. It is an approach without alternative to provide the genetic variability needed in the future.

Honeybees are kept by men, who, if the breeds do not meet the need of the beekeepers, will look for other breeds. Consequently, a key issue is to be informed on the apicultural demands of the beekeepers with respect to the colonies they want to work with. It is also important to consider the needs of the beekeepers in the breeding programs otherwise it will not bear any fruitful results. A multifunctional analysis of the questionnaire (which traits and their relative importance) of several hundred beekeepers will provide region-specific demands of the end users of colonies, which should be considered in the breeding program.

22.6 Main Threats

22.6.1 Deforestation

Sodhi et al. (2004) outline the depressing reality of deforestation in Southeast Asia. This region has the highest rate of tropical deforestation in the world, and is predicted to lose three quarters of its original forest and 42 % of its biodiversity in the next 100 years. The impacts of deforestation on honey bees are poorly understood. Liow et al. (2001) used honey baits to trap bees along transects in disturbed and relatively undisturbed sites in Singapore and Jahor in peninsular Malaysia. The proportion of Apidae (stingless bees and honey bees) was very low in oil palm plantations and very high in undisturbed sites.

22.6.2 Hunting

Asian people have been hunting honey bees for more than 40,000 years (Crane 1999) and bee hunting is still widely practiced throughout the region. Hunting *A. dorsata* and *A. laboriosa* is much more brutal, and often involves burning the bees with a smoldering torch of tightly bound brush (e.g., Valli and Summers 1988; Lahjie and Seibert 1990; Nath et al. 1994; Crane 1999; Tsing 2003). Some harvested colonies may be able to regroup, especially if the hunt occurs in daylight. Often, however, the hunt is conducted in darkness. The hunter bangs his torch on the branch supporting the colony to create a shower of sparks. The bemused bees follow the sparks to the forest floor (Tsing 2003; Oldroyd and Wongsiri 2006) where they crawl about, often with singed wings. Many queens are lost during these harvests, and their colonies perish along with them. The level of hunting pressure is most likely increasing in many areas. Finally, decreasing areas of forested land increases the hunting pressure on the remaining forested pockets (Nath et al. 1994).

22.6.3 Loss of Nest Sites

Cavity nesting bees require cavities for nesting. *A. cerana* is able to nest in manmade structures, or in the hollows of coconut palms (*Cocos nucifera*), and we think it likely that cavities are rarely a limiting resource. Nonetheless, Inoue et al. (1990) found that when *A. cerana* species nests in the small cavities of coconut palms, its growth is limited, and this may hinder its ability to produce reproductive swarms of viable size.

22.6.4 Parasites and Pathogens

Honey bee colonies can be affected by a variety of fungal, viral, and bacterial infections, and can be infested by various insect and mite parasites (Morse and

Nowogrodzki 1990; Bailey and Ball 1991; Oldroyd and Wongsiri 2006). Wild populations are not normally threatened by the parasites and pathogens with which they coevolved, and most wild colonies we have encountered are pictures of robust health. However, adverse effects of pests and diseases may arise when wild populations are stressed by environmental degradation. *A. mellifera* has been introduced into most Asian countries at one time or the other, almost certainly exposing wild Asian *Apis* to novel pathogens. Thus, the European foulbrood observed by Allen et al. (1990) may well have had its origins in the *A. mellifera* colonies introduced into Kathmandu by well-meaning but incompetent aid agencies. Since the 1980s, many populations of *A. cerana* have been severely infected by so-called Thai Sacbrood virus, which kills early pupal stages and is often lethal to colonies (Abrol and Bhat 1990; Verma et al. 1990; Nath et al. 1994; Chinh 1998; Abrol 2000). The origins of this pathogen are unknown, but it potentially arose from the anthropogenic movement of temperate strains of *A. cerana* into tropical areas, or from introduction of *A. mellifera*. European foulbrood is also known to originate from *A. cerana* (Bailey 1974).

A Conopid fly *Physocephala parralleliventris* Kröber (Diptera: Conopidae) parasitizes *A. cerana*, *A. koschevnikovi*, and *A. dorsata* in Borneo (Tingek et al. 2004). It grasps flying bees in flight and deposits a tiny larva on the integument. The larva penetrates the bee's cuticle, consuming the bee from the inside. An emerging threat to Asian *Apis* is the small hive beetle *Aethina tumida*. Originally from sub-Saharan Africa (Dietemann et al. 2009), this pest has recently spread to Australia, the USA and Egypt (Mostafa and Williams 2002; Neumann and Elzen 2004; Ellis and Hepburn 2006) where it causes significant damage, especially in warm, wet climates. The pest normally lives saprophagically on falling debris from a honey bee colony. Mostly, the bees confine the adult beetles to unreachable crevices (Ellis and Hepburn 2006). Occasionally, however, the beetles are able to overwhelm the host colony's defenses. The floor of the hive becomes a seething mass of beetle larvae, which apparently attracts more adult beetles. Within a day or so the larvae invade the brood comb at which point the colony will either abscond or be killed.

22.6.5 Climate Change and Forest Fire

The Intergovernmental Panel on Climate Change Fourth Assessment Report (2007) suggests that due to a 70 % increase in greenhouse gas emissions over the 100 years from 1906, the average temperature of the Earth has risen to 0.74 °C, and that this has decreased precipitation in parts of Southeast Asia. With expected increases in greenhouse gas emissions over the next two decades, global temperatures will most likely increase by a further 0.4 °C. In Southeast Asia, peak years for wildfire coincide with severe ENSO-induced droughts (Duncan et al. 2003), which are anticipated to occur more frequently with global warming. Drought combined with extreme wild fire events, and human impacts including deliberate fire setting associated with slash and burn agriculture (Brown 1998; Nath and Sharma 2007) are altering the structure of plant communities across the Asian region (Taylor et al. 1999).

22.6.6 Pesticides

Exposure to most insecticides kills individual foragers, and can kill whole colonies (Desneux et al. 2007). Some commercial fruit crops, particularly longan (*Dimocarpus longan*), litchie (*Litchi chinensis*), and citrus are major honey producers which are highly attractive to honey bees (Crane et al. 1984). Other orchard trees like the mangosteen (*Garcinia mangostana*) and rambutan (*Nephelium lappaceum*), make ideal nesting sites for dwarf bees (Oldroyd and Wongsiri 2006). These orchards are regularly sprayed with insecticides, which kills all colonies nesting in the tree canopy (personal observations). Spraying during flowering may also affect colonies nesting outside the crop but foraging in the crop. Some tree crops such as oil palm, *Elaeis* spp., are regularly sprayed, and this may contribute to the observed paucity of bees within oil palm plantations. Regulation of pesticide use is lax in some Asian countries, and this can increase the possibility of bee exposure to pesticides, for example, by contamination of streams.

22.6.7 Competition with Introduced *A. mellifera*

Concerns have sometimes been raised about the possibility that introduced *A. mellifera* may outcompete and displace indigenous honey bees in Asia (see for example, Verma 1991). We think this unlikely. Feral populations of *A. mellifera* are unknown in Asia, and in our view, are unlikely to be formed. First, in tropical regions with small variation in day length, European honey bees have difficulty regulating their rates of brood production and so they rarely reach swarming strength (Rinderer 1988). Second, wherever *A. dorsata* is endemic, its parasitic mite *Tropilaelaps clareae* is also present, and likely to infest any feral *A. mellifera* colonies and kill them. Even where *T. clareae* is absent, feral colonies are likely to be killed by *Varroa destructor*. Host shifts between *Varroa destructor* to *A. mellifera* are rare (Anderson and Trueman 2000; Solignac et al. 2005), and so indigenous *Varroa* are usually unlikely to infest *A. mellifera* colonies transplanted in to Asia. However, most *A. mellifera* populations worldwide, including transplanted Asian ones are already infested with *V. destructor*. Thus, establishment of a feral population from a domesticated one already infested with *Varroa* seems unlikely (Anderson 1994; Anderson and Sukarsih 1996; Oldroyd and Wongsiri 2006). Despite the foregoing discussion, it is clear that *A. mellifera* beekeeping has replaced *A. cerana* beekeeping in large parts of India, Japan, Pakistan, China, and Thailand, reducing population sizes of *A. cerana* in these regions. There is some evidence that very high densities of *A. mellifera* drones could interfere with *A. cerana* matings (Ruttner and Maul 1983) though in Japan at least the times of mating flights do not overlap (Yoshida et al. 1994).

22.6.8 Anthropogenic Movement

Only 10,000 years ago much of the Indonesian archipelago, the Andaman Islands, Taiwan, and Hong Kong were connected to mainland Asia (Heaney 1991). Rising

sea levels caused by the current phase of global warming created thousands of islands, some large, some small, and in doing so the once contiguous populations of honey bees were separated into isolated populations (Smith et al. 2000; Smith 2002; Oldroyd and Wongsiri 2006). This isolation has contributed to the rich diversity of honey bee ecotypes we see today, particularly in *A. cerana* and its related species (Hepburn et al. 2001; Radloff et al. 2005). Anthropogenic movement of honey bees between regions potentially erodes biodiversity by homogenizing the gene pool. For example, the ‘mainland Asia’ mitotype of *A. cerana* is ubiquitous across Asia, often alongside a regional mitotype (Smith and Hagen 1996; Smith et al. 2000). This suggests that humans have moved preferred strains of *A. cerana* among some of the islands of the South China Sea. Not only do such movements potentially reduce biodiversity, but can also spread pests, pathogens, and diseases.

22.7 What Should Be Done to Conserve Asian Honey Bees?

It is undeniable that forest clearing contributes to honey bee decline, and the cause of honey bees can only add to the chorus of plants and animals that are similarly afflicted. Clearing of old growth forest on this planet should simply be stopped. Nonetheless, conservation strategies must be rooted in pragmatism as well as good science, so we should also focus on those issues where something can be realistically achieved in the shorter term, and which will also be useful.

22.7.1 Quarantine

No doubt local people will continue to move *A. cerana* nests among neighboring islands, and there is little that can be done about this. Most of the ports and airports of Asia give priority to the free flow of goods and people believe that the economic benefits of doing so outweigh the potential costs to agriculture and the environment. However, there are some exceptions. Malaysia, for example, does not allow importation of *A. mellifera* into Borneo. South Korea and Japan have banned imports of queens and packages from countries where *A. tumida* is now endemic. We applaud these measures.

In view of this situation, research work on this bee species should target the following:

1. Recording the actual status quo distribution of *A. cerana* subspecies and ecotypes in its native habitat.
2. Testing races/ecotypes from different region under different environmental conditions to achieve information on their general and specific environmental adaptation and suitability for beekeepers’ needs.
3. Development and implementation of breeding programs to match the demands of the beekeepers, resistance to diseases, and adaptation to global warming.

4. Development of strategies and methods to preserve this endangered honey-bee species and its races; analysis of genotype–environment interactions and landscape genetics.

This requires a multidisciplinary approach including population genetics, molecular genetics, physiology, morphometrics, and practical bee breeding etc., to ensure a sustainable impact on the Asian honeybee populations from a quantitative and qualitative point of view.

The previously listed problems cannot be solved by uncoordinated or isolated activities in different countries, but need a multidisciplinary and multinational integrated approach. The preservation and improvement of Asian indigenous honeybee races is at present a pressing issue which requires immediate action.

At present, very little information is available on these different basic and applied biological aspects of *A. cerana* in comparison to *A. mellifera*. Such information lies scattered in the literature and requires scientific synthesis.

The research work should be carried out in different ecological zones of *A. cerana* habitat. Physical survey of each zone/site along with review of existing knowledge about genetic diversity of *A. cerana* is essential. It also requires the consent of different nodal agencies of each *A. cerana* country to execute this work plan.

In order to mitigate risk for *A. cerana* genetic diversity loss and ensure sustainable use of this bee species in natural and agricultural ecosystems, a module with the following work plan is submitted.

1. Investigations on identification of subspecies and geographic ecotypes of *A. cerana* by morphometric and molecular-based methods

The entire *A. cerana* habitat should be divided into different agro-ecological regions/zones. To ensure representative samples of the honeybee populations from each region, the entire distribution area of this native bee species needs to be surveyed. These samples should be subjected to a morphometric analysis (Ruttner 1987) and a molecular genetic analysis using approximately 25 microsatellites (Solignac et al. 2005). In addition, with the results of the behavior, resistance, and performance tests (as given under line item no. 2), these data provide a sound base for characterizing new *A. cerana* subspecies.

2. Study on behavior, resistance, and performance of colonies of the different populations under different environments

At each of the regions, unrelated colonies are sampled and distributed over these regions. Each of the population should be represented by at least four colonies from each region. From these colonies, honey production, aggressiveness, absconding and swarming behavior, and disease resistance should be observed. Such data help in estimating genotype–environment interaction and the general adaptation of the different subspecies to different environments. These tests are repeated with a second set of colonies in the following year. The results of the 2 years of testing are of key importance for choosing some of these subspecies/ecotypes as a base for a breeding program. Those subspecies/ecotypes which fit mostly to beekeepers' demands should be subjected to a breeding program (as given under line item no. 4) for areas which are at the moment populated with exotic *A. mellifera* colonies.

3. Study (survey) on the apicultural demands of the beekeepers at different locations within the distribution area of *A. cerana*

If the honeybee breeds do not meet the needs of the beekeepers, the beekeepers will look for other alternatives. Consequently, it is of great importance to know the apicultural demands of the beekeepers with respect to the traits and their relative importance in *A. cerana* colonies. If the needs of the beekeepers are not considered in the breeding programs, the program will not be sustainable. A multifunctional analysis of the questionnaire (which traits and their relative importance) provided to the several hundreds of beekeepers will serve as a useful guide to meet their demands and should be a part of the breeding program.

4. Design breeding programs for *A. cerana* to improve its different subspecies for their adaptation to the local environment and the demands of the beekeepers

Considering the results of the 2-year performance testing (as given under the line item no. 2 and 5) and survey of the beekeepers (as given under the line item no. 3), a protocol about the performance tests of honeybees for *A. cerana* should be developed. Depending on the magnitude of genotype–environment interactions and the number of different races, different breeding populations are established eventually with specific testing protocols. Data collection and data input into race specific databanks should be organized (see www.beebreed.eu as an example for *A. mellifera*). Data about the performance tests are then subjected to genetic evaluation using a BLUP (Best Linear Unbiased Prediction) model approach (Bienefeld et al. 2007). Results of the genetic evaluation should be publicized so that breeders and possible buyers of the queens make use of this information. Artificial insemination and the establishment of isolated mating areas for breeding colonies are further steps towards a sustainable genetic improvement program of *A. cerana*.

5. Developing performance tests for adaptation to hot and dry environmental conditions and resistance to Thai Sacbrood Virus disease

To efficiently develop tests for adaptation to hot and dry environmental conditions, we need solid information on the climate change to be expected within the distribution area of *A. cerana* for the decades to come. For these estimates, cooperation with a specialized institute for global warming is required. The observations should include tests on adaptation of individual worker bees and the colony as a whole towards hot and dry conditions.

The resistance tests to Thai Sacbrood Virus should be carried out at the research institutes in different countries of *A. cerana* origin. Two approaches, i.e., a hygienic behavior towards infested brood and a possible immune response of brood towards this virus need to be developed and tested for suitability.

6. Developing an education program for performance testing and queen rearing suited to the different socioeconomic conditions in the vast distribution area of the Asian honeybee

To be sustainable, the entire conservation program should be run by the beekeepers alone after an initial phase. This needs a sound experience and knowledge by the bee breeders during the initial phase with respect to performance testing of bee colonies, queen rearing, and using data from genetic evaluation for selection. In

addition, breeders need to be trained in artificial insemination of *A. cerana*. The training should also include practical courses and theory training in breeding and selection in honeybees. Suitable booklets and audiovisuals for such training also need to be developed.

7. Use of in situ and ex situ strategies for conservation of *A. cerana*

In some zones/areas, some subspecies may be detected with very limited population size or (at the moment) with negative economic traits to meet the needs of beekeepers. For such subspecies, in situ and/or ex situ conservation strategies are recommended and for this purpose, some reservoirs/protection areas in the distribution area of *A. cerana* need to be established. In such areas, single *A. cerana* races/ecotypes are freely allowed to multiply and migration of other subspecies/geographic ecotypes into such areas should not be permitted. This is an efficient (in situ) method to conserve genetic variability in small populations at low costs. In addition, conservation of all subspecies of *A. cerana* by an ex situ approach should also be planned. However, at present, techniques for long term conservation of semen and embryos in *A. cerana* are still lacking. There is a need to establish cryobanks for preservation of semen and embryos in different countries of *A. cerana* origin.

8. Zonation of beekeeping areas

In those countries of South and Southeast Asia where *A. mellifera* has already been introduced, beekeeping industry is passing through a dilemma because of competition for food, mating sites, and transfer of pathogens and parasitic mites. In order to resolve the above dilemma, zonation of beekeeping areas for *A. cerana* and *A. mellifera* beekeeping appears to be the only logical solution. Based on past several years of experience, it is now well documented that for subtropical region, *A. mellifera* especially the *ligustica* race is well suited and is performing well especially by foraging on intensive agricultural crops. In the temperate region, beekeeping with *A. cerana* should be encouraged because different subspecies/ecotypes of this native bee species are more prolific and superior genotypes than its counterparts in subtropical region and this native bee species forage efficiently on wild and scattered temperate flora. Such zonation of *A. cerana* and *A. mellifera* in different ecogeographic zones has been very successful in China, so India is also following the same strategy. This would greatly solve the problem of interspecies competition and both species could be complementary to each other.

9. Training and research center for Asian bees and beekeeping

For future development of beekeeping with *A. cerana* in South and Southeast Asia, a coordinated and systematic effort by establishing a training and research center for Asian bee and beekeeping in this region is essential. The overall objective is to generate and deliver improved beekeeping management technology through research and training primarily on Asiatic species of honeybee that will contribute to increased production and quality of different hive products (honey, beeswax, royal jelly, propolis, and venom) as well as better bee pollination service principally to the regional needs of South and Southeast Asian countries thereby ensuring a good source of income and nutritious food to rural poor communities

living at or below subsistence level. The center should assist different Government agencies, beekeeping communities, and commercial enterprises to create a cadre of beekeeping experts by training them in both practical and scientific aspects of beekeeping. It should provide information and advisory services and also act as a coordinating center for international cooperation in beekeeping and assist different developing countries of this region to establish a national program in beekeeping. Research needs to be carried out on Bee Biology, Bee Pathology, Bee Botany and Pollination, Beekeeping Technology and Equipment, Beekeeping Economics, Marketing Hive Products and Apitherapy. Training courses both in practical and scientific aspects of beekeeping may be offered for the benefit of beekeepers, beekeeping instructors, and beekeeping extension personnel from the department of agriculture, forestry, and rural development representing different beekeeping communities or associations, commercial enterprises, and Government departments from the entire region of South and Southeast Asia. International cooperation program will deal with technology sharing and technology transfer for the advancement of beekeeping in this region. It will organize regional training programs, workshops, seminars, conferences, and monitoring tours. It will also act as information dissemination center by publishing extension literature for the popularization and promotion of beekeeping and providing advisory services to individual participating countries and on regional basis also.

All the above programs may be carried out by keeping continuing contacts with national and international agencies. Establishment of Asian Apicultural Association (AAA) is a step forward in this direction and this organization should take initiative efforts for the establishment of training and research center for Asian bees and beekeeping.

22.8 Should We Encourage Keeping Native Honey Bees?

Clearly, if thousands of beekeepers each kept hundreds of colonies of a native honey bee like *A. cerana*, then the bee would be unlikely to go extinct. Should we therefore encourage keeping native *A. cerana* rather than European *A. mellifera*? The answer is 'it depends'. First, the benefits to beekeepers is to be considered. There is no doubt that *A. cerana* is resistant to parasites and pathogens are likely to be encountered, whereas throughout Asia, *A. mellifera* must be regularly treated to manage mite infestations. Furthermore, *A. cerana* can live happily in rough boxes or tree trunks with little or no need for expensive equipment (Oldroyd and Wongsiri 2006). On the other hand, there is no argument that in side by side trials, *A. mellifera* will always provide more honey, and provide a higher return on investment than can *A. cerana* (even if start up costs are higher) (Magsaysay et al. 2004). So if the capital is available, it is not really justifiable to encourage a less profitable form of agriculture. Poor beekeepers should not be expected to bear the burden of conservation, which is the responsibility of us all.

One important reason to encourage *A. cerana* beekeeping over *A. mellifera* beekeeping is that *A. mellifera* seems more vulnerable to predation by bee-eating birds than are the indigenous honey bees. Thus, some *A. mellifera* beekeepers in East Asia take steps to reduce bird predation by placing nets over apiaries. As many birds become entangled in the nets, there can be many bird deaths. In Thailand, much of the honey available in local markets is wild honey harvested from open-nesting species, and this seems to be preferred to bottled honey which is often of poor quality. Perhaps the best thing to do, then, is to encourage sustainable and hygienic harvesting of wild honey from dwarf bees, rather than encouraging a transition to *A. mellifera* or *A. cerana* beekeeping.

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Chapter 23

Livelihood Security

23.1 Introduction

Rural development aims to help people manage their livelihoods better through sustainable use of the available resources. It provides them with greater social and economic power by offering them opportunities to work in line with their capacity, without hampering the eco-services provided by their environment. Beekeeping and honey hunting have been practised by different societies since ancient times and have always been linked to development. ‘Honey hunting’—collecting honey from wild bee colonies—is an ancient practice as shown, for example, in cave paintings dating back to 11,000 BC found in Madhya Pradesh, India (Suryanarayan 2002), and in Ancient Egyptian drawings and paintings (Crane 1999). The history of beekeeping is rooted in and linked to honey hunting and associated practices. As settled farming became common, so too did the idea of keeping bees in hives, but beekeeping complemented rather than replaced wild collection. Gathering wild honey is still a common practice in many parts of the world; in India it is estimated that 22,000 tonnes of wild honey is collected by honey hunters annually—twice the amount of honey produced by the managed beekeeping sector (Wakhle and Pal 2000).

Different societies in Asia, Europe and North America have evolved their own beekeeping methods, investing in such diverse areas as bee genetics, hive design, management operations, managed pollination and honey processing and marketing. *Apis mellifera* as compared with Asiatic honeybee *Apis cerana* is a particularly fortunate bee species among hive bees. Scientists and development workers have studied it for more than 150 years, continuous selection and breeding have improved the bee’s genetics, and Langstroth’s hive design which is based on optimising bee space (the space required for the movement of bees) in the hive has helped beekeepers and bee enterprises to produce more honey in a sustainable manner. The mass introduction of Langstroth’s hive coincided with the start of large-scale application of pesticides in North American agriculture. This phenomenal shift in agricultural husbandry reduced pollinator diversity considerably, and farmers now felt the need to use honeybees as a source of pollination rather than just as a honey producer. Introduction of Langstroth’s hive also facilitated the large-scale transportation of bee

colonies for managed pollination, honey production, mass queen rearing and overall management of apiaries. Beekeeping has thus contributed to rural development through the centuries by supporting agricultural production, by providing honey, wax and other products for home use and by providing income for both farmers and the landless.

23.2 Beekeeping as a Business Enterprise and Market Potential

In nearly all countries of the world, bees and their products are not only well known and have wide consumer preference but also provide sustainable livelihoods to many small-scale farmers and other rural and non-rural people. Bees offer a large potential with minimal investments. As an agricultural enterprise, beekeeping does not require land ownership or rental, it can be started with equipment and tools that can be sourced locally, and in many instances, skills and knowledge required for such an enterprise are found within local traditions. As a business enterprise, it offers not only diverse products, for example, honey and wax among others, which can be sold in local markets and become an important source of regular income for farm families, but can also provide complementary services, such as crop pollination. Moreover, bee products improve farm family nutrition and can provide for traditional health care remedies.

Beekeeping is a lucrative trade even using simple management techniques, but local culture and economy need to be considered for it to be successful. Beekeeping as an enterprise fits in very well with small-scale farmers' livelihoods. It is not invasive; bees work along the natural patterns of local agro-ecological zones and provide positive impacts to the fauna and flora found within. It is an enterprise that can provide for employment, income and economic security for the farm family and others in rural areas. It requires little start-up investment, does not require complex technologies and techniques to start with and bees usually look after themselves, with little need for tendering. Bees provide for a plethora of products (honey, wax, pollen, royal jelly, propolis, venom, etc.) and are well known in many local markets. This provides a portfolio of products that a small-scale farmer can sell from a single farm enterprise. These products can also, with minimal processing, be 'transformed' into value-added products, for example, wax can be processed into candles and honey can be made into mead (honey beer).

The role of bees in sustaining livelihoods remains poorly known and appreciated. Bees are a fantastic world resource: they are essential for sustaining our environment because they pollinate flowering plants. Bees sustain our agriculture by pollinating crops and thereby increasing yields of seeds and fruits. The product that most people first associate with bees is honey, although beekeeping generates much more than just honey: the maintenance of biodiversity and pollination of crops are perhaps the most valuable services provided by bees. Honey is just one of several different products that can be harvested; others are beeswax, pollen and propolis, royal jelly and venom, and the use of bees in apitherapy, which is medicine using bee products.

Bees and beekeeping contribute to peoples' livelihoods in almost every country on earth. Honey and the other products obtained from bees have long been known by every society. The diversity in bee species, their uses and beekeeping practices varies greatly between regions. In many parts of the world, significant volumes of honey are today still obtained by plundering wild colonies of bees, whereas elsewhere beekeeping is practised by highly skilled people. Honey hunting of wild bee colonies still remains an important part of the livelihoods of forest-dependent people in many developing countries.

23.3 The Importance of Apiculture for Rural Livelihood

Beekeeping tends to be perceived as 'a hobby', or as 'a sideline activity'. These descriptions may often be true, but a resilient livelihood—one that keeps people out of poverty—is one that has access to range of options. In this case, apiculture and related trades can be sources of valuable strength to countless numbers of rural people's livelihoods. Rather than just a 'hobby', beekeeping may be seen as an important occupation and part of rural life worldwide. In rural communities where access to income is limited, small-scale beekeeping can contribute significantly to livelihood security. Apiculture and related trades tend to be underplayed in both policy and planning. One reason may be the focus of rural development, wherein crop production and livestock rearing are taken to be dominant activities in rural areas. This perspective can render invisible the part beekeeping occupies in social life, culture and local economies.

Beekeeping does not fit easily into the sectoral divides of rural development: as an activity, it spans forestry, horticulture, agriculture, the natural environment, animal husbandry and entomology without fitting precisely into any one of these sectors. Likewise, pollination is an important part of horticulture, yet the management of bees is often considered part of animal production. Similar problems confront the classification of bee products because honey is a food, yet beeswax is listed amongst non-food waxes and oils. Beekeepers have been categorised in different times and places as farmers, hunters and gatherers, cattle-keepers or rural dwellers—with beekeeping remaining hidden as an important skill and part of their lives. These ambiguities present complications for development policy makers, practitioners and researchers, even though such complexity is in keeping with the way people themselves link different activities, resources and products together in their daily lives. This very complexity explains the attraction of sustainable livelihoods approaches for securing a more visible position for beekeeping within rural development (Carney 1998). Beekeeping fits well into the people-centred perspectives of sustainable livelihoods approaches. Such approaches have contributed towards moving rural development away from economic and resource-based interventions, towards people and their rights and obligations to the resources on which their livelihoods are based. Beekeeping is a small-scale but very widespread activity. Unless you are aware of it, it is easy to visit villages and not see beekeeping. It does not attract much attention.

23.4 Creating a Livelihood from Beekeeping

According to the accepted definition originally developed by Chambers and Conway (1992): 'A livelihood comprises the capabilities, assets and activities required for a means of living. A livelihood is sustainable when it can cope with, and recover from, stresses and shocks and maintain or enhance its capabilities and assets, both now and in the future, while not undermining the natural resource base'.

Beekeeping is a useful means for strengthening and creating people's livelihoods because it both uses and creates a range of different capital assets. Successful beekeeping can be achieved by drawing upon all of the five categories of capital asset shown above, although financial capital need not be essential for productive beekeeping. (Although, of course, financial capital assets may be essential for family and household well-being, without which beekeeping may not be possible.) The five types of capital assets are a fundamental part of the framework used to explain the sustainable livelihoods approach.

Beekeeping does not attract much attention. It is easy to visit villages and not 'see' beekeeping, unless actively looking for it. Beekeeping, however, is crucially important for agricultural well-being; it represents and symbolizes the natural biological interdependence that comes from insects, pollination and production of seed. Useful small-scale efforts to encourage beekeeping interventions can be found throughout the world, helping people to strengthen livelihoods and ensuring maintenance of habitat and biodiversity.

Strengthening livelihoods means helping people to become less vulnerable to poverty. This is achieved by helping them to gain greater access to a range of assets, and supporting their capacity to build these assets into successful livelihood activities. In this chapter, how beekeeping can play in creating sustainable livelihoods is discussed. People who have limited cash or financial savings often have other assets or strengths—as opposed to needs—that can be mobilized.

23.5 Beekeeping Assets

Individual livelihoods depend on access to many types of assets which fall into five categories: natural, human, physical, social and financial. To understand this, think about your own livelihood and all the diverse assets you depend upon: your skills, access to transport, equipment, telecommunications and the social networks you have been born into or have created yourself. No individual category of capital assets, such as finance, is sufficient on its own to create a livelihood. Beekeeping is a useful means of strengthening livelihoods because it uses and creates a range of assets. Successful beekeeping draws upon all categories of capital assets, although financial capital is *not* essential for getting started in productive beekeeping.

23.6 Natural Capital

Livelihoods depend upon natural resource stocks: in the case of beekeeping, these are bees, flowering plants and water. Bees feed on the nectar and pollen from flowers; the nectar is eventually converted into honey. Bees also collect gums and resins from plants and use plants and trees as habitat for nesting places. Bees are a natural resource, freely available in the wild. Bees colonise where they can, so wild or cultivated areas, wasteland and even land-mined areas all have value for beekeeping. Beekeeping is possible in arid areas and places where other crops have failed: the roots of nectar-bearing trees may still be able to reach the water table far below the surface. Beekeeping is therefore feasible in marginal conditions, just the sort of activity that is needed where people have to restore their livelihoods or to create new ones.

Beekeeping provides an excellent bonus crop in addition to, but not instead of, other crops. Bees are the only livestock capable of harvesting nectar and pollen. There is no competition with other animals, and without bees, these valuable resources would not be harvested. The extra-remarkable aspect of beekeeping is that it ensures the continuation of natural assets: by the pollination of wild and cultivated plants. As bees visit flowers, they are not only collect food for today but also, by their pollinatory activities, ensure future generations of food plants, for future generations of bees as well as for us, the perfect self-sustaining activity.

Beekeeping fits well alongside many other livelihood activities and the natural resources used by them (for example, forestry, agriculture, conservation activities). Although impossible to quantify, pollination is the most economically significant value of beekeeping. Flowering plants and their associated bees are interdependent: you cannot have one without the other. Referring to the definition of a livelihood, that it can enhance its capabilities *‘while not undermining the natural resource base’*, it is clear that beekeeping actually helps to sustain the natural resource base. How many other income-creating activities can be said to restore natural resources? Beekeeping has been in the past a regular part of village agriculture worldwide, and we need to ensure that it is retained as farming practises change.

23.7 Human Capital

Traditionally, many societies have good skills relating to bees, honey and making other products. Often the products of beekeeping are used by women in making secondary products. For example, the important industry of honey wine making (locally referred as *Tej*) in Ethiopia is run by women, and elsewhere in Africa it is often women who brew and sell honey beer. These are the types of human skills needed to create livelihoods within a society. Many beekeeping projects have ignored existing skills, or worse, implied that they are wrong or out-of-date. The best projects recognise existing skills and build on them for greater income generation and ensured sustainability.

23.8 Physical Capital

These include the infrastructure (transport, water, energy, communications, buildings) and the production equipment that enable people to make their livelihoods from beekeeping. Frame hive beekeeping is used in all industrialized countries, and many beekeeping projects have tried to introduce this type of beekeeping. However, where a society does not have the physical, human or financial assets to support this type of beekeeping, the project is likely to fail. There continue to be projects introducing beekeeping technology that cannot be sustained. Yet, there are many ways of managing bees and obtaining a crop from them. A hive is just a container for bees to live inside, and there are many types of such containers. To achieve sustainable beekeeping projects, all equipment must be made and mended locally, and in the process, equipment manufacture contributes to the livelihoods of other local people. Indeed, beekeeping can stimulate many different sectors within a society: village traders, carpenters (making hives and stands), tailors (making veils, clothing, gloves), container-makers and sellers. The equipment needed for beekeeping can be very simple, for example, the humble plastic bucket is one of the most useful items. For the beekeeping expert, it may not bring great professional kudos, simply to recommend the provision of good quality, lidded, stackable plastic buckets. Yet, these are essential items for beekeepers living in remote places who need to keep their honey clean until they are able to sell it. Excellent quality honey can be harvested as long as clean buckets are available, along with cotton or baskets for sieving honey, and containers for melting wax and for selling the honey and other products. Infrastructure such as transport and roads can be critical for beekeepers in remote places: access to transport brings access to marketing possibilities and better prices.

23.9 Social Capital

Social resources such as networks, producer and marketing associations are of great significance for beekeeping development. Such associations provide the means for beekeepers to advance their craft, ensure protection of their bees, processing for honey and wax, access to markets, and marketing support. Access to a network at a wider level, as provided by Bees for Development, assists beekeepers to make contact with national and international networks, to find out about sources of training, markets, research findings, and raises their awareness of the industry and available opportunities.

23.10 Financial Capital

Access to finance is essential for the further development of beekeeping enterprises; for example, successful marketing depends upon the purchase of containers for processing and packaging of products. Credit is necessary for beekeeping associations

running collection centres, buying products from producers and selling in bulk. However, significant financial assets are not essential for beekeeping at subsistence level. A good beekeeping project will work to ensure that all available capital assets are taken into consideration, without dependence on any that are not. For example, too many projects have depended on the importation of the beeswax foundation used in frame hives; this is impossible for beekeepers without financial assets.

23.11 The Sustainable Livelihoods Approach

The sustainable livelihoods approach allows appreciation of how these capital assets fit into the sustainable livelihoods framework. The framework assists with consideration of the various factors that constrain or enhance the livelihood of a beekeeper and his or her family. In the framework shown later, the understanding of sustainable livelihoods is separated into five parts: the vulnerability context; people's livelihood assets; policies, institutions and processes; livelihood strategies and livelihood outcomes.

23.12 Vulnerability

The framework considers people living and working within a context of vulnerability. Analysis of vulnerability means to identify the risks beekeepers are under and the resilience they have to cope with negative change in their environment, both short- and long-term. Vulnerability includes shocks, trends and seasonality. Shocks could be hurricanes damaging agriculture and destroying honey harvests or the arrival of a new bee disease. Trends may be the gradual decline in the quantity of flowering plants due to habitat loss or gradual increase in demand for honey. Vulnerability may be also seasonal; for example, a beekeeper's family may have less food at the beginning of the rainy season, making them more vulnerable to illness, and with less time for beekeeping. People's access to assets, and their capacity to utilise them, is shaped by their resilience to negative shocks, trends and seasonality.

We see situations worldwide where beekeeping can be especially valuable, as it remains an activity possible for people living in even the most difficult of circumstances, isolated by war or sanctions. This is because bees are nearly always available in the wild and equipment can be made from whatever is to hand.

For rural development projects, use of the sustainable livelihoods framework can help to identify the ways in which people are most vulnerable and how they are strongest. This may lead to suggestions of how to make them stronger, for example, by helping them to diversify into beekeeping activities. It may also help a beekeeping project to identify ways for government and donors to reduce vulnerability, for instance, by providing training to cope with the effects of a disease of honeybees, or to prevent the use of insecticides.

23.13 Livelihood Assets

Analysis of people's access to assets is based on the idea that they require a range of assets to achieve positive livelihood outcomes. This part of the framework has been already discussed earlier, in relation to the assets needed for beekeeping.

23.14 Policy, Institutions and Processes

The policy, institutions and processes part of the framework includes organizations large and small institutions, legislation and the processes that link organizations, institutions and policies to people's lives. These have a profound influence on people's access to assets. They shape people's livelihoods and effectively influence the following:

- Access to various types of capital assets, to livelihood activities (such as beekeeping), and to decision-making bodies and sources of influence.
- The terms of exchange between different types of assets (for example, making it difficult to market honey because traders lack access to credit).
- The returns (economic or otherwise) achievable from any given livelihood strategy.

Policy, institutions and processes also have a direct impact upon whether people are able to achieve a feeling of inclusion and well-being. Looking at the framework, it can be seen that there is feedback from policy, institutions and processes to the vulnerability context. This is because policies established and implemented by organizations affect trends directly and indirectly. They can also help cushion the impact of external shocks, for example, a change in legislation affecting world trade in honey. Institutions influence people's choices of livelihood strategy, and policies and regulations often affect the attractiveness of particular livelihood choices (for example, legislation concerning honey marketing or making export difficult).

23.15 Livelihood Strategies Involving Bees

People's capacity to make a livelihood, and their resilience to negative change, is shaped by their *livelihood strategies*. These strategies are the combination of people's activities and the choices they make in order to achieve their livelihood goals. They depend on the opportunities and access individuals, households and communities have to exploit different levels and combinations of assets, and are probably the major influence on people's choice of livelihood strategy. For example, in a household that depends on farming for most of its food and income, one person may decide to take up beekeeping, and in time, this may provide the capital for another to start a small shop. The beekeeper's success will depend on the available opportunities:

maybe there will be a friend who keeps bees. If the friend encourages the beekeeper to join an association, this may be a good opportunity (an example of how social capital works). The possibility to start depends also upon the beekeeper having access to suitable capital assets (human, social, natural and physical), such as tools and equipment, a safe place to keep the hives, and a means of learning (from the friend) how to keep bees.

23.16 Apiculture's Role in Poverty Alleviation

When apiculture forms part of people's livelihood strategies, there are various possible outcomes. Some of these outcomes will include income and material goods as well as non-material outcomes such as well-being and contentment. In terms of apiculture, the least visible livelihood outcome is the pollination of flowering plants, both wild and cultivated; this is an outcome impossible to quantify. Honey is a traditional medicine or food in nearly all societies and whether sold in a simple way at village level or packaged more sophisticatedly, honey generates income and can create livelihoods for several sectors within a society. Beeswax is also a valuable product from beekeeping, although in some places, its value is not appreciated. Industrialized countries are net importers of beeswax, and the supply comes from developing countries.

The beekeepers and other people in a community can create further assets by using honey and beeswax to make secondary products, such as candles, beauty creams or beer. Selling a secondary product brings a far better return for the producer than selling the raw commodity. Bees also generate other products (pollen, propolis and royal jelly) that can, in some situations, be harvested, marketed and made into secondary products, all of this work effectively strengthening people's livelihoods.

Another crucial livelihood outcome is where, through strengthening people's livelihoods, beekeeping has managed to help a family become less vulnerable, strengthening their ability to look into the future and reducing the chance that they will slip into poverty if a member of the family becomes ill or if a season is bad for farming or other activities. In addition to their financial value, honey and beeswax have many cultural values and form part of ceremonies for birth, marriages, funerals, Christmas and other religious celebrations in many societies. Beekeepers are generally respected for their craft. All of these aspects are livelihood outcomes from the activity of beekeeping. While some may be difficult or impossible to quantify, they are real outcomes that strengthen people's livelihoods and therefore should be acknowledged by a beekeeping intervention. Beekeeping will help improve crop productivity, and it has a social aspect also in improving the socioeconomic status of the farming community by offering many of the products as well as a source of income through ancillary industries.

23.17 The Value of Honeybee Pollination to Agriculture

Pollination by honeybees is as vital to the production of many crops as water and sunlight. There is no substitute! One-third of our daily diet relies on honeybee pollination. Almonds, apples, sweet cherries, plums and prunes are examples of crops that require cross-pollination between varieties in order to produce a crop. Bee pollination is necessary for the production of cucumbers, squash, pumpkins and melons. Fruit and nut crops are known to produce larger yields when pollinated by honeybees. These fruit, nut and vegetable crops have a value approximately 35 times greater than the income generated directly by the beekeeping industry.

The greatest value of honeybee pollination is associated with the production of seeds that will have worldwide distribution. Most of the vegetables, including asparagus, carrots, celery, onions, radishes and turnips produce seeds only when their flowers have been adequately pollinated. Likewise, seed production of forage crops, such as alfalfa, various clovers, trefoil and vetch, requires many visits by foraging bees. Including the 'indirect' value of honeybee pollination (meat, dairy products, vegetables, hay, etc.), honeybee pollination is really worth in excess of 400 times the intrinsic earning power of the bees to beekeepers.

23.18 Value of Honeybees to Non-agricultural Segments

Besides the fact that honeybee pollination is responsible for producing most of the planted vegetable and flower seeds, home gardeners should realize that honeybees are necessary to their gardens, much like water and sunlight. Without honeybees, fruit trees bear few fruits, berries tend to be small and misshapened and vine crops like melons, cucumbers, squash and pumpkins bear small fruits that do not fill out and mature properly. Some ornamental shrubs and trees also require pollination to produce fruits that may be eaten by birds or other beneficial animals. To assure adequate pollination of fruit trees and garden crops, gardeners are going to have to encourage beekeeping in their communities.

23.19 Wildlife and Watershed Management Areas

Drastic reductions in populations of native insect pollinators have created a great need for honeybee pollination to insure re-seeding and perpetuation of wild plants. These plants serve as sources of fruits, nuts and/or vegetation for consumption by various birds and mammals. They also provide nesting sites and hiding places for other creatures involved in the intricate balance of nature. This vegetation also adds immeasurably to soil conservation and flood control. Too often, honeybees are equated

with stinging, a suicidal act reserved specifically for purposes of colony defence. Frequently, the insects behaving in an aggressive manner at picnics and around homes are wasps (visiting for meat) that are incorrectly called bees.

23.20 Crop Productivity

Beekeeping contributes to economic security in another way, through the positive effect on pollination in agriculture in the rural areas of developing countries. Even though pesticide use is still on the rise in modern agriculture, managed pollination has been able to make up for certain pollinator deficiencies and has increased productivity and thus incomes. Tables 23.1, 23.2, 23.3 and 23.4 illustrate the importance of managed pollination and the economic value that pollination can have for agricultural and horticultural crops. In places like the USA, Canada, Europe and Japan, honeybees have long been used for the pollination of crops like apples, almonds, pears, plums, cucumbers, melons, watermelons and berries. Honeybees were first used for pollination in the USA in 1895, when *A. mellifera* honeybees were used to pollinate pears in Virginia. However, the Himalayan region still lags far behind in making use of honeybees for crop pollination, with the first reports of colonies of honeybees being used for pollination coming from Himachal Pradesh in India, where they were used for apple pollination in 1996 (Partap and Partap 2002).

In the early 1990s, the worldwide annual contribution of pollinators to the value of agricultural crops was estimated to be US\$ 54 billion. Honeybee pollination alone accounts for an estimated US\$ 15 billion in crop production in the USA (Table 23.4). Similar estimates have been made for other countries (Table 23.4). The increase in income of poor farmers from honeybees also contributes to the success of rural development efforts and activities.

23.21 The Role of Pollination in Improving Food Security and Livelihoods

For a farmer, the most desired goal in agriculture is to get the maximum possible crop yields and better quality fruits and seeds under given inputs and ecological settings. It is particularly important to get a premium price for the produce when farmers are engaged in cash crop farming. There are two well-known methods for improving crop productivity. The first method is making use of agronomic inputs, including plant husbandry techniques, such as the use of good quality seeds and planting material, and practices to improve yields, for example, providing good irrigation, organic manure and inorganic fertilizers and pesticides. The second method includes the use of biotechnological techniques, such as manipulating rate of photosynthesis and biological nitrogen fixation. These conventional techniques ensure healthy growth of crop plants, but work up to a limit. At some stage, crop productivity becomes

Table 23.1 Impact of honeybee (*A. cerana*) pollination on fruit productivity (Himachal Pradesh, India, and Kathmandu Valley, Nepal) (Partap 2002)

Crop	Increase in fruit set (%)	Increase in fruit weight (%)	Increase in fruit size (length, diameter) (%)
Apple	10	33	15, 10
Peach	22	44	29, 23
Plum	13	39	11, 14
Citrus	24	35	9, 35 (premature fruit drop decreased by 46 %, juice increased by 68 % and sugar content in juice increased by 39 %)
Strawberry	112	48	Misshapen fruits decreased by 50 %

Table 23.2 Impact of honeybee (*A. cerana*) pollination on vegetable seed production (Kathmandu Valley, Nepal) (Partap 2002)

Crop	Increase in pod set (%)	Increase in seed set (%)	Increase in seed weight (%)
Cabbage	28	35	40
Cauliflower	24	34	37
Radish	23	24	34
Broad leaf mustard	11	14	17
Lettuce	12	21	9

Table 23.3 Average increase in crop production from honeybee pollination (Soldatov 1976; cited in Free 1993)

Crop	Increase in production (%)
Alfalfa	65
Buckwheat	39
Coriander	35
Cotton	28
Cucumber	11
Cucurbits	25
Flax	35
Grape	29
Linseed	19
Rape	30
Red clover	82
Sainfoin	60
Tree and bush fruit	35

stagnant or declines with additional inputs for the known agronomic potentials of crop will have been harnessed (Partap and Partap 1997).

The third and relatively less known method of enhancing crop productivity is through managing pollination of crops using friendly insects, which in the process of searching for food perform this useful service to farmers (Partap and Partap 1997).

Table 23.4 Economic value of honeybee pollination

Country	Estimated Economic Value
USA	US\$ 15 billion
Canada	US\$ 1.2 billion
EEC	US\$ 300 million
New Zealand	US\$ 2,253 million
China	US\$ 0.7 billion

Pollination is an ecological process based on the principle of mutual interactions or inter-relationships (known as proto-cooperation) between the pollinated (plant) and the pollinator. Pollinators visit the flowers of the plants to obtain their food (i.e. nectar and pollen) and in return pollinate them. In many cases, it is the result of the intricate relationship between plants and its pollinators, and the reduction or loss of either affects the survival of both. In recent years, the Convention on Biological Diversity (CBD) has recognized pollination as a key driver in the maintenance of biodiversity and ecosystem function.

The pollination process involves the transfer of pollen from the male part of the flower called ‘anthers’ to the female part called ‘stigma’ of the same flower (self-pollination) or another flower of the same or another plant of the same species (cross-pollination). Pollination is vital for completing the life cycle of plants and ensuring production of fruits and seeds, whether agricultural crops or natural vegetation/flora. This ecological process is an essential prerequisite for fertilization and fruit/seed set. If there is no pollination, there will be no fertilization, no fruits or seeds will be formed and farmers will harvest no crop. Pollination is therefore the most crucial process in the life cycle of the plants and is essential for crop production and biodiversity conservation and helps enhance farm income and rural livelihoods. Figure 23.1 shows the relationship of pollination to improved livelihoods through enhancing agricultural productivity and biodiversity conservation.

Many cash crops are actually self-sterile and require cross-pollination to produce seeds and fruits (McGregor 1976; Free 1993). But it is not only self-sterile varieties that benefit from cross-pollination but self-fertile varieties also produce more and better quality seeds and fruits if they are cross-pollinated (Free 1993). Logically, the increase in the cultivation of cross-pollinated cash crops will also increase the need for managed pollination. Equally interesting is the adoption of apiculture as a new enterprise by many people. Promoting use of beekeeping for pollination of cash crops will be of benefit to both the beekeeper who will receive money for the pollination services of his/her honeybees and harvest honey and to the farmer whose income will be increased through boosting crop productivity as a result of pollination services of bees. This will help ensure food security and enhance the livelihoods of both the farmers and the beekeepers (Fig. 23.1). This system of hiring and renting honeybee colonies for apple pollination is being practiced in Himachal Pradesh in northwest Indian Himalayas. In Maoxian County in the Hengduan Mountains, a somewhat similar but rather unsustainable system of apple pollination is prevalent. Here, farmers hire ‘human pollinators’ for pollinating apple and pear trees by hand.

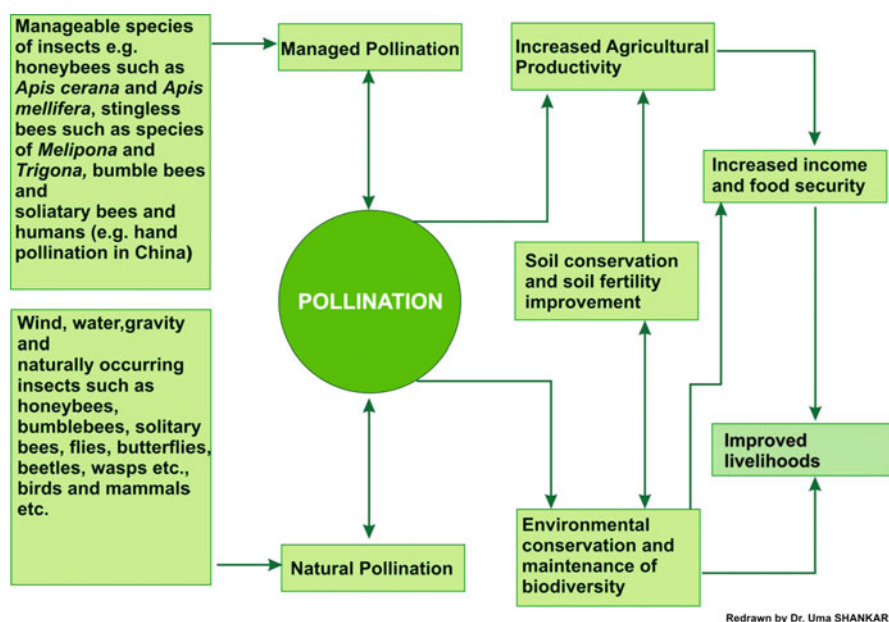


Fig. 23.1 Contribution of pollination to agricultural productivity and improving rural livelihood

23.22 Inadequate Pollination as a Factor Affecting Crop Productivity

The ongoing transformation from subsistence to cash crop farming poses new challenges for maintaining crop productivity and quality. There are signs that across the Hindu Kush Himalayan (HKH) region, the overall productivity of many mountain crops is going down. Possibly the worst affected crops are the cash crops like fruits, particularly apples, and off-season vegetables that are the hope of the region in terms of providing farmers with cash income and underpinning development efforts. This reduction in productivity is taking place despite extensive efforts at extension and information to support improvements in a range of management practices and strong support for the introduction of successful commercial varieties. The studies revealed that among the several factors affecting mountain crop productivity pollination plays an important role. Evidence of this emerging pollination problem has been documented in a series of field studies carried out by The International Centre for Integrated Mountain Development (ICIMOD) across the Himalayan region (Partap 1998; Partap and Partap 2000, 2001; Partap et al. 2001). These studies investigated the state of inadequate pollination, its cause factors and its impact on crop productivity.

23.23 Pollinator Diversity and its Role in Enhancing Crop Productivity

Pollinators provide an essential ecosystem service that contributes to the maintenance of biodiversity and ensures the survival of plant species including crop plants. Two types of pollinators occur in nature. These include abiotic pollinators such as wind, water and gravity and biotic pollinators such as insects, birds and various mammals. It has been estimated that over three-quarters of the world's crops and over 80 % of all flowering plants depend on animal pollinators, especially bees (Kenmore and Krell 1998). Globally, the annual contribution of pollinators to the agricultural crops has been estimated at about US\$ 54 billion (Kenmore and Krell 1998) (Table 23.2).

Insects are the most commonly occurring pollinators of many agricultural and horticultural crops. Different kinds of insect pollinators such as bees, flies, beetles, butterflies, moths and wasps are important pollinators of many crops. Among insects, bees are more effective pollinators than other insects because, unlike other insects, they are social and collect nectar and pollen not only to satisfy their own needs but also to feed their young; their body hair help transfer pollen from one flower to another; they show flower constancy and move from one flower to another of the same species; and many species can be reared and managed for pollination.

Over 25,000 species of bees are found in the world. These include honeybees, bumblebees, stingless bees and solitary bees. Bees are the most effective pollinators of crops and natural flora and are reported to pollinate over 70 % of the world's cultivated crops. It has also been reported that about 15 % of the 100 principal crops are pollinated by domestic bees (i.e. manageable species, e.g., hive-kept species of honeybees, bumblebees, alfalfa bees, etc.), whereas at least 80 % are pollinated by the wild bees (Kenmore and Krell 1998). These non-honeybee pollinators are estimated to provide the pollination services worth US\$ 4.1 billion per year to the US agriculture (Prescott-Allen and Prescott-Allen 1990). There are number of industries that can be based on beekeeping to generate income for farmers/rural communities. These industries are referred to as ancillary industries.

23.24 Beekeeping and Ancillary Industries

An ancillary unit is defined as a unit which produces parts, components, sub-assemblies, and tooling for supply against known or anticipated demand of one or more large units manufacturing/assembling complete product and which is not a subsidiary to or controlled by any large unit in regard to the negotiation of contracts for supply of its goods to any large unit. This shall not, however, preclude an ancillary unit from entering into an agreement with a large unit giving it the first option to take formers output

23.24.1 Industries Necessary for Apiculture

23.24.1.1 Supply Industries

There are a number of specialist industries which operate solely (or predominantly) to supply requisites to the beekeeping industry. While not quantified as a part of the beekeeping industry, their existence is certainly a part of the indirect economic impact generated by the industry. This 'supply' sector includes

- 1. Beehive manufacturers.** While only small in number, companies manufacturing hives for the industry tend to specialize in this activity.
- 2. Extractor/uncapping machinery.** Any company skilled in the production of stainless steel equipment for the food industry would be capable of manufacturing the machinery required to 'uncap' combs and extract honey.
- 3. Packers' equipment.** Bottles and bottling equipment are required by packers. In addition, drum manufacturers provide the special galvanized drum, made with side bung that is not generally available from other sources for producers and packers. A gradual change to plastic drums is taking place, with intermediate bulk containers, of pallet size being used which hold more honey than the traditional drums.
- 4. Heat source.** Every honey producer requires a steam or hot water boiler to generate steam, and hot water, for processing honey and wax. While not requiring a specialized manufacturing activity, the beekeeping industry generates a demand for such equipment.
- 5. Transport/handling equipment.** All commercial beekeepers must purchase trucks and utilities for transporting and servicing hives. Many also own front end loaders for loading hives, on pallets, and loading drums or use other forms of mechanisation.
- 6. Other equipment.** Beekeepers also have a need for other equipments such as electric generators and mobile extracting units. While the first two items (as for heat sources) do not require a specialized manufacturing activity, expenditure by the beekeeping industry can provide an important source of demand.
- 7. Quality assurance.** Beekeepers and packers are increasingly introducing quality-assured premises and equipment for handling honey as a food product for human consumption. Again, this is not necessarily a specialised activity unique to the industry, but represents an additional demand for existing services.

It is clear that a considerable amount of other economic activity is generated as a result of the activities of the honeybee industry.

23.24.2 Industries Dependent on Apiculture

Major products of beekeeping are honey and beeswax. In addition, specialized segments of the industry concentrate beekeeping activities towards the production of

queen bees and package bees—the provision of specialist paid honeybee pollination services for horticultural and agricultural industries. Queen bees and package bees are sold to other beekeepers, both within the country and overseas. Honey and beeswax are still produced by queen and package bee producers, but these products are not the prime goal of their beekeeping activities. Other minor products from the industry include pollens, royal jelly, propolis and bee venom. The economic dimensions of the industry and the direct and indirect impacts of beekeeping are as follows.

23.24.2.1 Industry by Sector

The overall apiary industry should be considered in terms of a number of sectors, as follows:

1. Honey

Honey is the prime output of commercial beekeepers, and it is produced by bees from plant nectar. The major producers are Russia, China, USA, Mexico, Argentina, Canada, Brazil and Australia. The major exporters are China, Mexico and Argentina, but the highest colony yields are recorded in Australia and Canada, which have a favourable environment as well as highly developed colony management. The major consumers and importers are the industrialized countries led by Germany, Japan, USA and UK. The increased consumption over the last few years can be attributed to the general increase in living standards and a higher interest in natural and health products. Western Europe as a whole imported approximately 140,000 tonnes which is about 55 % of the consumption. The average European Union per capita consumption of 600 g per year varies widely amongst individual nations, from Greece with 300 g per capita to Germany with 1,800 g per capita.

In general, light-coloured honeys bring the highest price and dark ones are most frequently used for industrial production. Mild-flavoured honeys are preferred, but characteristically flavoured honeys bring top prices in some countries. Large honey packers usually prefer honeys with a low tendency to crystallize. Some unifloral honeys such as Hungarian black locust honey bring twice the price of regular, multifloral honey. Small shipments into Switzerland of unifloral honeys such as lavender honey, in most cases already bottled, bring much higher prices. Local prices in most developing countries are higher than the international market prices, and prices in neighbouring countries with less honey production or favourable exchange rates *may* sometimes be quite attractive. Expansion of markets with honey-containing products should be considered on a national level or for across-the-border trade. Consumer education and of course, spending power will probably be the most important factors influencing the possibility of expanding local markets or for increased product diversity. The examples given in this chapter may serve as ideas for possible modification and adaptation to individual circumstances.

2. Beeswax

Beeswax is a substance secreted by the worker bees. It is recovered by beekeepers primarily from honeycomb cappings, and also from cull combs and wax pieces. Beeswax is used in certain pharmaceutical and cosmetic preparations, as a base for polishes and some ointments, for candles, and for comb foundation for beekeeping. It has the highest melting point of natural waxes, and can be sold in either raw or refined form. Commercial beeswax is generally refined for sale by a manufacturer of apary products.

The cosmetics and pharmaceutical industries have no complete substitute for beeswax. At least small quantities will always be needed to maintain quality and specific characteristics. Like honey prices, prices for beeswax may vary considerably from place to place.

Markets and prices for products made from beeswax vary widely from country to country. In these industries, beeswax forms only a minuscule part of both the manufacturing process and the final product. It is used in candle making, skin creams, *grafting wax for horticulture*, polishes and varnishes, paste furniture polish, liquid furniture polish, spray polish, floor polish, shoe polish (cream type), crayons, leather preserves, waterproofing textiles and paper, paint, wood preservative, swarm lure, veterinary wound cream and adhesive lotions.

3. Live bees

The production of queen bees, and of entire colonies of bees, is the main diversification available to beekeepers. The queen bee industry is dependent on the existence of a profitable honey industry and on an export market to buy queens at a period when there is little or no sale. For example, in Australia the demand for queen bees is estimated at around 155,670 per annum. At an average price of \$ 9/queen, this represents a farm-gate value of around \$ 1.5 million. This is a conservative figure because export sales—estimated by industry sources to be \$ 0.75 million—are not recorded separately and have not been added. Live bee exports is a potential growth area for the beekeeping industry, as further markets develop.

Package bees and nucleus colonies are other forms of live bee production and are sold both within the country and overseas. Again, data on total value of production for this sector of the industry are not available and it has been estimated on the basis of known production. The total value of this sector has been assumed to be \$ 2.25 million, which is almost certainly an under-estimate, but which has been used as a conservative minimum. In India where great potential of beekeeping exists, marketing for live bees can be much more.

4. Other products

In addition to honey and wax, active beehives are also a source of other products. These include:

Royal jelly It is a milky white smooth jelly secreted by nurse bees and used to feed developing queen larvae and young worker bee larvae. The production of royal jelly is a very specialized procedure, and flora conditions must be ideal before production

can be considered. Royal jelly is used as tablets or mixed into creams and shampoos. Royal jelly can be sold in its fresh state, unprocessed except for being frozen or cooled, mixed with other products or freeze-dried for further use in other preparations. The fresh production and sale can be handled by enterprises of all sizes, as no special technology is required. In its unprocessed form, it can also be included directly in many food and dietary supplements as well as medicine-like products or cosmetics. For larger industrial scale use, royal jelly is preferred in its freeze-dried form, because of easier handling and storing. Freeze-dried royal jelly can be included in the same products as the fresh form. The production of freeze-dried royal jelly requires an investment of at least US\$ 10,000 for a freeze-dryer, sufficient production volume and an accessible market for the raw material or its value added products. Products containing royal jelly should be specially marked or packaged in order to distinguish them from similar products without it.

As a dietary supplement Royal jelly belongs to a group of products generically described as 'dietary supplements'. These are products which are consumed not for their caloric content or for pleasure, but to supplement the normal diet with substances in which it may be lacking.

As an ingredient in food products A mixture of royal jelly in honey (1–3 % royal jelly) is probably the most common way in which royal jelly is used as a food ingredient. Among the advantages of this product are that no special technology is required and the honey masks any visible changes in the royal jelly. The final product is pleasant-tasting and it provides the beneficial effects of both products.

As an ingredient in medicine-like products In medicine-like formulations, royal jelly is generally included for its stimulatory effects. However, it is also used to solve specific health problems. A variety of formulations are available, often containing ingredients otherwise used to alleviate particular afflictions or as medicine.

As an ingredient in cosmetics Except in Asia, probably the largest use of royal jelly is in cosmetics. Royal jelly is included in many dermatological preparations, but mostly in those used for skin refreshing and skin regeneration or rejuvenation. It is also used in creams or ointments for healing burns and other wounds.

Others The only other known uses for royal jelly are in animal nutrition. In particular, royal jelly has occasionally been used (fresh or freeze-dried) to stimulate race horses. For experimental purposes, it is also used as a food for rearing mites and insects.

Royal jelly collection Royal jelly is produced by stimulating colonies to produce queen bees outside the conditions in which they would naturally do so (swarming and queen replacement). It requires very little investment but is only possible with movable comb hives.

Propolis It is a by-product of the bee hive. It originates as a gum secretion gathered by bees from a variety of plants and can vary in colour depending on the plant species of origin. Propolis has remarkable therapeutic qualities and is much sought after in some countries for the treatment of a range of human ailments and for

cosmetic purposes. It is used by honeybees as an antiseptic to varnish the interior of honeycomb cells used by the hive to rear young brood, to seal cracks in the hive from the winter chill, and for general hive cleanliness purposes. The market for raw material and secondary products containing propolis will probably continue to grow as they find more acceptance in medicinal uses and as more cosmetic manufacturers realize their benefits and marketing value.

Bee venom It is collected by stimulating bees with a mild electric current. The venom used in the preparation of pharmaceutical materials. It can be used to detect hypersensitivity or allergic reaction to bee stings. Bee venom is a highly specialized product with very few buyers. The market volume is relatively small too, although there are no comprehensive surveys. The main venom producer is the USA, which has produced only about 3 kg of dry venom during the last 30 years (Mraz 1982), but there is a large producer in Brazil and more or less significant amounts are produced in many other countries. Prices in 1990 varied greatly between US\$ 100 and US\$ 200 per gram of dry venom (Schmidt and Buchmann 1992). Prepared for injections or sold in smaller quantities, prices can be much higher. However, the beekeeper often does not get this price. The prevailing prices in European and Asian markets are generally slightly lower.

Pollen Pollen can also be harvested by beekeepers, at a rate of around 7–10 kg per hive per year. Pollen is used by bee colonies as a source of protein, but harvesting pollen by the beekeeper requires detailed knowledge of resources, hive management, species flowering variations and timing, and hive response to different honeys and pollens. Pollen is collected via specialized traps fitted to the hives and must be processed rapidly after collection (usually via freezing or drying) to avoid excessive moisture absorption and fermentation. Many beekeepers harvest pollen to feed back to their hives during periods of natural pollen deficiency. Dried pollen prices in the USA range from US\$ 5 to 13/kg wholesale and from US\$ 11 to 30/kg retail (*American Bee Journal*, 1993). Encapsulated pollen or pollen tablets are sold in vials of 50–100 units and retailed at prices of up to US\$ 900/kg, at least in Italy. The bulk pollen consumer market seems to be growing in industrialized countries, but pollen tablets are still a common feature of health food stores and command an excessively high price. Encapsulation and extraction of pollen lend themselves easily to small-scale manufacturing and result in safer consumer products.

Most of the buyers and large scale sellers of pollen are also honey traders. Crane (1990), however, reports that a lot of commercial pollen is not bee collected but machine-collected from certain wind pollinated plants which release very large quantities of dry pollen. At least in industrialized countries and those with increasing numbers of health conscious consumers, pollen consumption is likely to increase further. On the other hand, there seems to be a wide market for reasonably priced, encapsulated pollen and tablets.

5. Paid pollination services

Some beekeepers receive payment for placing hives in close proximity to flowering crops, according to contractual arrangements with farmers. For example, rates for pollination services in inland Australia varied between \$ 25 and \$ 35 per hive in 1996, with variations between crops. It has been estimated that at least \$ 2.9 million is received by the industry in this way, based on total payments received for pollination services in Tasmania (Gifford 1989) and multiplied up to an Australian figure by numbers of hives.

Similar concept is picking up throughout the world, including India. In Himachal Pradesh, India, this practice has already started and is likely to be followed in other states as the awareness about pollination benefits is realized by the farming community.

Evidently, to ensure the country's self-sufficiency in foodstuffs and to receive foreign currency from excess production, the stabilization of rural populations by complementary activities of both a financially rewarding and environmental nature is necessary, and there is no doubt that beekeeping fits perfectly within this framework. Hence, efforts are required to popularize and increase beekeeping, and an enormous potential is still waiting to be tapped.

23.24.3 Value of *A. cerana* Beekeeping for Mountain People

Mountain areas are characterized by inaccessibility, fragility, marginality, diversity, niche and adaptation mechanisms (Jodha 1990). *A. cerana* beekeeping system fits well with these characteristics and supports the livelihood of mountain people. Several mountain areas are inaccessible and lack transport and communication infrastructure, thus in these circumstances, migratory beekeeping with *A. mellifera* becomes highly expensive, vulnerable and a high-risk business. Stationary beekeeping system with *A. cerana* is more suitable and fits well in mountain farming systems and processes. Mountains are fragile environments, and the productivity in these areas is hampered by uncertain rainfall, low fertility and lack of agriculture inputs. Cycle of negative changes keeps on hitting the mountains, which results in a non-conducive situation for *A. mellifera* beekeeping, as it requires more ideal conditions for economic returns. On the other hand, *A. cerana* can live under these adverse conditions; even if everything goes wrong and colonies abscond, the farmer does not lose anything, as bees reoccupy their hives when conditions allow them to do so (Table 23.5).

Mountain farmers are used to living on margins, hence their livestock, including honeybees (*A. cerana*), has also evolved to adjust in these circumstances. *A. cerana* proved its credibility to survive, thrive and produce in highly marginal conditions. People living on margins do not have the resources and information to keep themselves updated and to meet emerging demands of livelihoods. It is very difficult for deprived mountain people to cater to the survival requirements of exotic *A. mellifera* (Table 23.5). Borders between agro-climatic zones in mountain areas are fluid and depend upon changing weather phenomenon. These agro-climatic zones lack

Table 23.5 Comparative advantages of *A. cerana* over *A. mellifera*

Parameter	<i>A. cerana</i> (Asian)	<i>A. mellifera</i> (European)
Initial investment	Very low	High
Colony management costs	Negligible	High
Risk involved	Low	High
Potential for stationary beekeeping	Suitable	Not suitable
Susceptibility to mites and predators	Resistant	Susceptible
Eco-services	High	Low

vastness but are rich in biodiversity. Diversity in *A. cerana* populations in different nesting areas has been evolved from the time immemorial, which evolved to an enormous strength to catch up with the difficult mountain-specific circumstances. It is reflected in its behaviour while passing through the abrupt flash raindrops, which occur in mountain valleys without any notice, when bees are on foraging spree. Its foraging behaviour has also been evolved, keeping in view the diversity of floral resources. Its capacity to fight, escape and adjust with the parasites and other enemies is also commendable. Bringing a honeybee species from far away distances and adopting it into completely different environs is really an uphill task for poor mountain farmers. The qualitative niche and comparative advantage of native honeybees under mountain-specific condition can be explained in terms of honey quality, its organic background and its acceptability. Mountain communities have adapted themselves to the harsh mountain realities. Indigenous knowledge in this respect helps them to sustain the difficulties of mountain environment. *A. cerana* beekeeping is an integral part of their indigenously evolved understanding about nature, diversity and practices. People evolved management skills with minimum labour and resources to maintain Asian hive bee culture since centuries. *A. cerana cerana* of Jumla, Nepal, was introduced in low-lying Kathmandu valley, and this introduction was found to be successfully fruitful. Jumla bees have adjusted the rhythm of their life in seasonal environmental and nectar flow regime changes.

The use of honeybees in agriculture is a well-known technique to improve crop production. In addition to increased yield, the quality of the product will improve as a result of fully pollinating the flower. An apple requires up to five foraging trips before becoming fully fertilized. Bees are efficient pollinators because of their behaviour, known as foraging consistency, in which they only work on one plant species per trip. A bee visits hundreds of flowers during each trip and makes about ten trips a day. If placed near an orchard, the bees will consistently pollinate the orchard during its specific bloom. As long as the food source is near, the bees will pollinate only the desired plants in the orchard. The bees are then moved to another location to match different bloom times.

Once established, the Asian honeybee does not tolerate moving the hive. The Asian honeybee is only for stationary beekeeping. Studies show the Asian honeybee is a more efficient pollinator than the European honeybee. Crop yields are higher using the Asian honeybee. The Asian honeybees operate at lower temperatures, hence they begin pollinating earlier than the European honeybee. This is critical in areas where temperatures are very low, especially in almond-growing areas where blooming

begins in March. The Asian honeybee is more effective in pollinating key crops and can pollinate a higher variety of plants. With smaller hives and colonies, the Asian honeybee requires less forage for survival. The European honeybee colonies are larger and produce a large quantity of surplus honey, whereas the Asian honeybee colonies are smaller, producing less honey. The foraging range of the Asian honeybee is one half of that of the European honeybee. This means it covers only a quarter of the area. However, the range of the European honeybee exceeds the requirements of most villages. The Asian honeybee adequately covers a village and surrounding areas.

The Asian honeybee *A. cerana* is well-suited for small-scale stationary operations. It is economical at any scale because of the small initial investment, simple equipment requirements and negligible operating costs. The Asian honeybee is a more efficient pollinator than European honeybee, resulting in greater increases in village income through pollination services. One estimate cited by UC-Davis claims \$ 14 benefit for every \$ 1 invested due to increased production. The Asian honeybee is native to the region and tolerant to pests and diseases such as mites and wasps that destroy the imported European honeybee. The equipment is simpler, smaller and less expensive than that for the European honeybee. By using simple designs such as the Japanese box pile hive, villagers can locally reproduce the hives, which is easier than reproducing the hives using standard European bee equipment. The Asian honeybee can sustain itself even when orchard crops are not blooming, by foraging in the surrounding area for desert flowering plants. The Asian honeybee is known for its ability to survive and thrive in harsh, marginal conditions.

However, there are some weaknesses with *A. cerana* beekeeping. The Asian honeybees have a smaller foraging range and are ill-suited for migratory beekeeping. They produce less honey per hive, but the honey is considered more valuable in overseas markets. The Asian honeybees cannot be raised near areas where European honeybees are used, as they will raid the honey from the European hives.

The Asian honeybee is the traditional honeybee used by beekeepers, particularly in the mountainous areas. Therefore, Asian honeybees are recommended as an alternative option that is economical for sustainable apiculture. It restores traditional practices and is well-suited for the environment. The Asian honeybee provides more efficient pollination. This will significantly improve rural income through better yields and improved quality of key agricultural products. The Asian honeybee is well-suited for small-scale, village-level rural development. The European honeybee is still relevant. However, its use should be concentrated on developing large-scale, migratory, commercial or cooperative operations.

23.24.4 Promoting Secure and Sustainable Livelihoods Through Beekeeping

Beekeeping has holistic benefits that relate to health (being used as a medicinal product and as food), the economy (directly through sales of honey and other bee products,

and indirectly through increased productivity of pollinated crops, as well as bee enterprise activities), employment (in honey production and pollination services) and the environment (by ensuring pollination of wild species). Beekeeping can contribute to securing sustainable livelihoods by transforming vulnerabilities into security, an idea incorporated in ICIMOD's Strategic Plan 2003–2007 (ICIMOD 2002). It can be carried out by small farmers, and is particularly suitable for under-privileged, landless and low-income groups as well as women, as it requires minimal start-up investment and generally yields profits within the first year of operation. Some of the different roles played by beekeeping in rural development are described in more detail and discussed in the following pages.

23.24.5 *A. mellifera and Indigenous Himalayan Bees*

Beekeeping with *A. mellifera* has become an important part of many beekeeping initiatives and programmes from north to south. These initiatives facilitate technology transfer and ensure the constant supply of honey to the world markets. However, as a part of this endeavour, *A. mellifera* has been introduced and promoted in areas beyond its original natural nesting habitat. In the Himalayan region, there are four indigenous honeybee species, one of which (*A. cerana*) can be kept in hives. These bees have special advantages for farmers at the higher altitudes where they are found naturally. However, the advantages of honeybee biodiversity remained unrecognised for many years, and little was done to conserve the indigenous honeybee resources. As a result of lack of information and ignorance, efforts including beekeeping as a component in rural development were sometimes counterproductive. The benefits of beekeeping depend on ensuring that the approach is locally appropriate, and in some areas, this will mean that beekeeping should focus on indigenous species.

23.24.6 *Physical Security*

The link between beekeeping development and the physical security of societies and communities may not seem immediately obvious, but it does exist and is based on the pollination services that bees provide. As with all pollinators, bees from both managed apiaries and the wild play an important role in combating soil degradation by enhancing the replenishment cycle: more pollination, more seed sets, more plants, more biomass returned to the soil (Ahmad et al. 2003), leading to less soil erosion, less flooding, and a more conducive environment for sustainable living. Pristine areas play a pivotal role in maintaining the replenishment cycle by conserving and absorbing water, obstructing and regulating flash-floods, disseminating important plant and weed seeds for regeneration and providing a habitat for a large number of plants and animal species. Wild and feral bees (domestic bees that have

escaped to the wild) play an important role in pollinating flowering plants in pristine areas, hence increasing the vitality and viability of these physically secure environments.

The Himalayan cliff bee *Apis laboriosa* is one of the most important pollinators in high-altitude pristine areas of the Himalayas. It nests at high altitudes and can fly long distances at heights where many lowland birds and insects have difficulty breathing. This bee not only pollinates a significant number of plant species at high altitude but also provides food for the local fauna. Monkeys feed on bee brood; wasps, hornets and some bird species eat bees; bears are known to have a soft spot for honey; and lizards also eat fallen bees.

23.24.7 Conservation of Resources Through Bee Diversity

The natural environment can only be maintained in a healthy state through the interest and active involvement of local people. Beekeeping is a good way for people to earn an income without damaging the environment. At the same time, honeybees and other pollinators play an important role in the conservation of plant resources by providing pollination services. These services also support diversification, which is necessary for the process of evolution. Many times, conservation of wild flora is an 'unnoticed' activity that happens under the cover of bush, canopy, and the darkness of the forest; pollinators participate by supporting the gene flow, which is a vital process of life. Resource conservation is an important aspect of rural development activities and it also includes development programmes for bees like the Asian hive bee *A. cerana* and other wild honeybee species. These activities and programmes mainly aim at conserving bee resources in a way that serves both poverty alleviation and biodiversity conservation. ICIMOD runs one such programme based on conservation apiculture, which focuses on improving the productivity of the Asian hive bee through selection and multiplication. Efforts are being made to involve beekeeping communities: farmers and beekeepers receive economic and social incentives to participate in the selection and multiplication activities. The communities have clearly benefited from this programme. At one of the project sites in Nepal, the number of farmers and beekeepers in the project has increased, and the selected bee colonies produce more honey. The Indian butter tree forest also benefited from this programme as social fencing provided by local beekeeping communities discouraged irresponsible logging of this important bee tree.

Pollination deficits and the need for more livelihood options often encourage farmers and entrepreneurs to rear butterflies and moths along with bees. This unique enterprise has a potential for growth as more people become involved in raising these beautiful insects for income generation and conservation. The conservation aspect of this effort is very strong, with people's attitudes towards these important pollinators changing rapidly from elimination to rearing and conservation. The growing trend in this enterprise has brought the natural beauty of these insect pollinators to people's

homes in different forms, and market forces have capitalised on their unmatched beauty.

23.25 Economic Security

Beekeeping can help economically vulnerable communities achieve economic stability. Honey production, pollination services, agriculture, and forestry are but a few of the economic benefits of beekeeping. Bee products such as propolis, royal jelly, beeswax and bee venom are also high-value, low-volume green products. In addition to the direct income from bee products, beekeeping generates off-farm employment opportunities in many fields, including hive carpentry, honey trading, renting and hiring of bee colonies for pollination and bee-based microenterprises.

23.25.1 *Honey and Other Bee Products*

Poor farming communities and landless farmers in the South have welcomed the introduction of the European honeybee *A. mellifera* and adopted this bee species as a source of inspiration and a way to alleviate the ills of poverty. Countries in South America, South and Southeast Asia, Central Asia and Africa have started producing enormous quantities of honey, honey markets have expanded and the demand for bee products has increased (Fig. 23.1). Central America, Mexico and the Caribbean alone host 3.5 million bee colonies with an average honey production of 24.7 kg/hive—about 9 % of world honey production (Crane 1990). In Central America, most honey is produced by small beekeepers. In the Yucatan Peninsula, for example, more than 17,800 small beekeepers from the Maya community produce about one-third of Mexico's honey (Arce Arce and van Veen 1997). World honey production increased from close to 900,000 tonnes to nearly 1,400,000 tonnes between 1975 and 2005. Many of the benefits from the increased honey production have gone to the rural poor who were directly involved in the production, contributing substantially to rural livelihoods, even though major portions of the benefits from marketing and scaling up have gone to traders and managers of large operations. Higher incomes in developed countries have opened up the markets for honey and other organic bee products. A more organic lifestyle has changed the diet of the rich and prosperous, and the demand for honey for the table, bakery and meat processing has increased tremendously over the last 20 years. In addition to this, beauty and health care products based on beeswax and propolis are becoming more popular. Cosmetics and pharmaceuticals account for approximately 60 % of total bee product consumption. Beeswax is used for candles, cosmetics, pharmaceuticals, polishing materials, as a component of modelling waxes and as a glazing agent for food products. It is also a release agent, stabiliser, texturiser for chewing gum base, carrier for food additives

(including flavours and colours) and a clouding agent. In 2005, 8,000 tonnes of beeswax was consumed in the of the European Union countries alone.

23.25.2 Value Chain for Economic Security

Competition in the honey sector is getting fiercer, and several stakeholders have started to use a value chain approach, especially to gain access to organic markets. A value chain can be defined as a sequence of productive processes from the provision of specific inputs for a particular product to primary production, transformation, marketing and distribution, and final consumption. Honey is a major organic product and is being scrutinised by buyers and other actors in trade and marketing.

A value chain systematically takes all steps of a production process into account. It analyses the links and information flows within the chain and reveals the strengths and weaknesses (and even losses) in the process. It also analyses the boundaries between national and international chains, takes into account buyers' requirements and international standards and allows international benchmarking (Richter 2005). The value chain approach addresses the so-called critical success factors that determine whether a product meets market requirements with regard to quality, price, dependability, volume, design and speed of delivery, and consequently improves competitiveness. Value chains generally include three or more of the following: producers, processors, distributors, brokers, wholesalers, retailers and consumers. The partners in the value chain work together to identify objectives; they share risks and benefits; and they invest time, energy and resources to make the relationship work. The value chain approach is an actor-oriented approach and is very effective in tracing product flows, showing value-adding stages, and identifying key actors in the chain and the relationships between them (Schmitz 2005). In the past, most honey and bee-related projects were only active at a particular level of the value chain. They mainly focused on the promotion of beekeeping and the production of honey, rather than on the delivery of a product in a competitive market. The concept of a value chain approach dealing with the whole process is fairly new and there are only a few organisations engaged in scaling up beekeeping by using a value chain.

Beekeepers, packers and producers may find it difficult initially to adapt to this approach, as the beekeeping industry in the developing world is not really prepared for following the requirements of a value chain. But with growing awareness and capacity, many stakeholders are increasingly using value chains to achieve economic security. China, Brazil, Argentina and Mexico are adopting a similar approach to improve competitiveness and the quality of bee products to harness the benefits of honey trade. The value chain map shows the flow of honey and other bee products in the market and the distribution of income from consumers to beekeepers and input suppliers. In many cases, it is difficult to find clear vertical lines between each level in the chain. Many beekeeping entrepreneurs and honey traders act as integrated value chain operators and perform two or more functions in the chain. The same company, cooperative or organisation acts as a service provider (training and technical inputs),

beekeeper (maintains apiaries), honey processor and trader. In order to improve the economic situation of the rural poor, government agencies and many international organisations are supporting rural farmers to make use of locally available resources to produce commodities for income generation. The value chain map reveals that in beekeeping, most of the supporting agencies focus more on the promotion of beekeeping itself than on products for processing and marketing. However, experience from Nepal suggests that producing commodities alone does not help rural farmers or producers if they cannot sell their products or if there is little value added at their end of the value chain. It is equally important to link rural producers with markets and sustain and grow these links so that they form a perpetual growth cycle of production and consumption. Connecting rural producers with markets on a sustainable basis is a very challenging task that can be helped by value chain promotion. Globalisation has brought unique opportunities for developing countries in terms of access to markets for their products.

However, in order to benefit from these opportunities, these products must be competitive in global markets. Value chain promotion helps to develop systemic competitiveness by looking at the whole chain of production activities and strengthening the overall production chain. To enhance the competitiveness of the commodities and generate more income, it is essential to strengthen linkages between value chain operators. Value chain supporters can make a greater impact if they plan intervention strategies and facilitate the implementation of activities in close cooperation with the various stakeholders in the industry.

23.25.3 Empowerment

Empowering communities and societies is one of the major prerequisites for achieving secure and sustainable livelihoods. Beekeeping can play an important role in empowering the poor, and it also introduces the concept of fair and equitable sharing of benefits in societies. Beekeeper communities understand the structure of a beehive, where life organises itself in a more meaningful and disciplined way. Beekeeping in a community inspires people to organise and work collectively for their common benefit and to trade their product in a systematic way. Empowered communities are able to demonstrate their economic and social power. Most small beekeepers belong to the more disadvantaged groups in society and it is important for development workers and projects to help them achieve better economic returns for their hard work, which in turn will contribute to their empowerment.

23.25.4 Extension

Organisation and mobilisation are a central part of community empowerment and help communities to access resources and achieve economic prosperity. ICIMOD's

programmes in Nepal have not only helped beekeeping but also helped honey hunting communities to organise themselves and to understand better the importance of cliff bee resources. This has led to the relative stabilisation of cliff bee (*Apis laboriosa*) populations, better income through the establishment of 'bee watch tourism' and improved eco-services like pollination for their crops and overall biodiversity management. There are many other examples from managed beekeeping. The Yucatan Peninsula was mentioned earlier; here, more than 17,800 small Maya beekeepers produce about one-third of Mexico's honey output and earn a significant amount of money (Arce Arce and van Veen 1997). In Pakistan, some very poor sections of society adopted beekeeping in the 1980s, and reports indicate that their overall economic situation changed within the first 10 years. They organised themselves and were eventually able to manage 400,000 bee colonies. Honey sales and exports expanded into the niche markets of the Gulf countries, bringing prosperity to these Pakistani beekeepers.

Beekeeping in Nepal is still at a preliminary stage, and most beekeepers keep *A. cerana* colonies in log and wall hives without any management except honey harvesting once or twice a year. Beekeeping also includes protecting wild bees and bee cliffs as part of the family or community ownership. This means that most of the honey produced in Nepal, both from wild bees and backyard beekeeping, is 'organic'. According to Neupane (2003), there are a total of 145,000 colonies of honeybees in Nepal including 110,000 colonies of the Asian hive bee *A. cerana*, 15,000 colonies of *A. mellifera*, and 20,000 colonies of other wild bee species. The Beekeeping Development Section (BDS) of the Ministry of Agriculture, quoted by Apinet-Nepal in 2006, recorded some 124,000 colonies of *A. cerana*, three-quarters of them kept in traditional fixed comb wall or log hives. Managed and modern beekeeping with *A. mellifera* is more commonly practised in the central and western development regions of Nepal.

Women in developing countries tend to be disadvantaged, and beekeeping can provide them with a way of improving their position. In Ghana, Africa, the position of women beekeepers changed with changes in the beekeeping business. Now women beekeepers control the cash flow and post-harvest processes of honey, including the trade in bee products. In other words, beekeeping has given them a chance to prosper and become empowered (Science Publishing House Kwame Sarkwah Aidoo 1997).

Capacity building can provide an important route to empowerment. In the HKH region, ICIMOD's capacity building programme on indigenous honeybees triggered a chain of events in empowerment. More than 6,000 poor men and women were trained in *A. cerana* beekeeping, which changed the art of beekeeping in many project areas. Beekeeping activities increased the communities' direct cash income by 25 % on average, and there were further benefits from pollination in terms of agricultural productivity and eco-services. Women's income in the different project areas increased, which provided them with better opportunities for health care, nutrition and education, also supporting empowerment. Some 27 % of the women benefited from the capacity-building programmes of the project in the region. The poorest of the poor in particular were trained to organise themselves better, and the setting up of cooperatives and grass root organisations and management of small grants

and endowment funds triggered the growth of the beekeeping industry through the establishment of hive carpentry and other bee-based enterprises.

23.25.5 *Social Security*

The definition of social security depends on the level of social and economic development of communities and nations. In the developed world, pension schemes, health care and insurance policies give people a sense of security. In developing countries, social security has a different meaning for different people and communities. In most cases, the rural poor rely on livestock, a piece of land or their beehives or bee colonies in the nearby forest to provide them with a sense of social and economic security, as cash flow is not reliable and often inaccessible. In these situations, beekeeping development is also integrated into rural development efforts.

In southern China, special efforts are being made to conserve local and indigenous honeybee species. A comprehensive conservation and development programme for *A. cerana* has been initiated, which is facilitating the conservation of 780,000 colonies of *A. cerana* in Yunnan province alone. Ethnic and other communities keep these honeybees in log, wall and moveable frame hives. As a result of regular selection and breeding programmes, the bee species in this area produces an average annual yield of 10 to 15 kg of honey, 1 to 2 kg of pollen, and 1 kg of wax per colony. In the best-case scenario, honey production of *A. cerana cerana* can reach 90 kg per colony (Kuang et al. 2002). This example shows clearly how beekeeping development using indigenous resources can contribute to social, economic and environmental security.

23.26 **Beekeeping as a Contribution to Improved Rural Livelihoods**

We have discussed how beekeeping contributes in a balanced way to rural development efforts, leading to secure and sustainable livelihoods. The major gains from beekeeping development efforts originate from agricultural husbandry, replenishment of forest resources and human satisfaction, bringing economic benefits to people, particularly to the poorest of the poor. Below we suggest a few approaches that should be incorporated in future rural development strategies for achieving the goal of sustainable livelihoods (Figs. 23.2, 23.3 and 23.4).

1. Sustainable policies to regulate the import and export of bees and bee products and control the spread of diseases between continents, countries, and within bee species
2. Improvement in trade policies to remove trade barriers and facilitate cross-border and cross-continent trade of bee products and to enhance the understanding of the value chain at all levels
3. Harmonisation and adoption of international honey quality standards



Fig. 23.2 Livelihood security training to farmers



Fig. 23.3 Livelihood security training to students

4. Incorporating managed pollination through honeybees as an important input in agricultural husbandry to be strictly followed by the concerned government departments
5. Improvements in training curricula to include conservation apiculture as an important element of beekeeping development; this change will provide space and opportunity to understand the holistic honeybee phenomenon, which includes all bee species including *A. mellifera*
6. Enhancement of nectar- and pollen-producing plant species in reforestation and urban beautification projects for the promotion of beekeeping



Fig. 23.4 Beekeeping by (a) a handicap, (b) women and (c, d and e) children

7. Encouraging the beverages and bottling industry to promote honey-based drinks for people's well-being
8. Development of a database on the nesting habitats of wild honeybee species for the promotion of green businesses like bee watch ecotourism
9. Awareness-raising campaigns for conserving wild honeybee habitats through public and private partnership

Nevertheless, in the context of large unrealized potentiality of rural beekeeping, the following socio-economic benefits can be achieved:

1. Promotion and enhancement of agricultural production.
2. Enhancement of the quality and production of fruits.
3. Promotion and expansion of forest wealth.
4. Increase in plant community in the environment.
5. Saving and/or earning foreign currency by producing and/or exporting honey and other bee products.
6. Prevention of diseases by taking pure honey regularly.
7. Cure of some particular diseases.

8. Promotion and increase in the nutrition value of food.
9. Use of wax and other bee products in various industrial products.
10. Promotion of medicine quality.
11. Upgrading the quality and standard of food in the view point of taste and nutrition.
12. Increase in the rural-based cottage industry in the country.
13. Family solvency through additional income broadly in the rural areas.
14. New employment generation by way of rural beekeeping extension.
15. Acceleration of the development of national economy.

23.27 Conclusion

Beekeeping is a vast scientific subject related to agriculture, food, nutrition, medicine, industrial products and environment. Asian countries have a large unrealized potential for the production of honey, wax, crops, fruits and other bee products in the field of beekeeping. There is a unique opportunity for rural development through the promotion and extension of beekeeping in these countries. As 80 % of total population of the country lives in the rural areas and depend on agriculture, beekeeping is a proven and profitable venture requiring less capital and skilled labours with high-yield enterprise in comparison with other poverty-reduction activities. Nevertheless, for rural development, beekeeping can play a vital role as one of the economic activities for one home on farm programme.

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About the Book

This book provides complete information on all aspects of *Apis cerana* beekeeping. The book is first of its kind which deals in details on biology, biogeography, reproduction, genetics, molecular phylogeny, interaction with other species, floral resources, dance language, safety from pesticides, management problems, loss of genetic diversity, behavioural defense, role in food production, livelihood security, and conservation strategies for protecting biodiversity and enhancing crop productivity. Despite its economic usefulness, biodiversity of Asian hive bee *Apis cerana* is suffering precipitous decline and is threatened with extinction in its entire native habitat. To promote beekeeping as a sustainable option for rural development, crop production, and bio-diversity conservation, there is an urgent need to generate information on this important species. Although a number of publications have appeared on honeybees in the market, no attempt has been made to approach the subject in a systematic and comprehensive manner in case of *Apis cerana*. The aim of this book is to fill the gap by providing detailed information on different aspects of *Apis cerana* leading to sustainability and environmental protection. The compilation of this book is unique in the sense that in the context of pollinator decline over the world, conservation of this species will be a step for sustaining food security.

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